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Genetics of Flea Beetle (*Phyllotreta cruciferae*) Resistance in the *Brassicacea*

By

©

Jane De Pauw

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for
the degree of Master of Science

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Genetics of Flea Beetle (*Phyllotreta cruciferae*) Resistance in the *Brassicacae*" submitted by Jane De Pauw in partial fulfillment of the requirements for the degree of Master of Science.

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Abstract

Sinapis alba is a valuable source for introgression of insect resistance into canola (*Brassica napus* L.). This research attempted to identify genetic sources of flea beetle (*Phyllotreta cruciferae*) resistance within a hybrid *Brassica napus/Sinapis alba* population and determine the genetic basis of this resistance using molecular markers. In total, 66 cDNA/genomic markers were positioned on 18 linkage groups in the initial development of a genetic map of the *Brassica napus* double haploid population designated as 98-B-4. Analysis for whole seed quality parameters and cotyledon fatty acid profile indicate that flea beetle resistance may be linked to seedling fatty acid quality elements as opposed to whole seed quality components. This research illustrates the potential to introgress DNA from the wild relative *S.alba* into *B.napus* and the value that this material may have in transferring novel genetic traits into modern cultivars of canola.

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I. Introduction

1.1 Importance of Canola

Canola (*Brassica napus* L.) is one of the principal oilseed crops grown in Canada with 4.87 million hectares of production in 2000 (DeClercq and Daun, 2000). The United States recorded 592, 220 hectares harvested in 2000 and in Europe 4.87 million hectares were harvested (FAO, 2000). In Europe, canola is referred to as Oilseed Rape. The main canola producing regions of the world are China, Canada, the Indian subcontinent, and Northern Europe. Since 1990, the canola/oilseed rape crop has been the world's third most important edible oil source (Downey, 1990). Plant breeding advancements have brought novel and expanded market opportunities for rapeseed oil. Since the introduction of canola to farmers in the early sixties, growth of the economic potential of the crop has meant more agricultural acres committed to canola production. As the area of canola production has expanded, problems caused by diseases and insects have also increased (Downey, 1990).

Throughout its growing period, canola is susceptible to attack from a variety of insects. The seedling stage is vulnerable to attack by flea beetle (*Phyllotreta cruciferae* Goeze, *Phyllotreta striolata* F.), red turnip beetle (*Entomoscelis americana* Brown) and two species of cutworms (*Euxoa ochrogaster* Guen and *Agrotis orthogonia* Morr.). Bertha armyworm (*Mamestra configurata*), diamond back moth (*Plutella xylostella* L.), root maggots (Delia species) and aphids (*Brevicoryne brassicae* L.) reduce yield potential from flowering to pod development. The Canadian canola crop had a farm cash value of 1.77 billion dollars in 1999 and therefore a significant pest outbreak can result in tremendous financial losses.

Flea beetle infestations have significant economic impact. Yield losses in the range of 10% are common where flea beetles are plentiful, even when pesticides and cultural farming practices are used to protect the crop. The average yield loss for canola production could be as high as 50% without chemical control of flea beetle (Palaniswamy, 1996). In the Northern United States, a one percent yield reduction results in a total crop loss of approximately 25 to 35 million dollars (Sustainable Agriculture Facts, 1997). Annual crop losses due to flea beetle damage, in North America are estimated to exceed 300 million dollars. Light to moderate infestations of flea beetles can delay plant development and result in uneven maturity. Delayed maturity in turn can expose the canola crop to adverse temperatures during the flowering period or to frost before the plants have matured. Variable maturity at harvest reduces seed quality and yield. Delaying harvest to allow immature pods to ripen results in yield loss due to shattering of the over-ripe pods prior to and during harvest. Harvesting too early also produces a crop with a high level of immature seeds and results in excessive chlorophyll levels, again affecting seed quality, yield and marketability. Protecting the canola crop from flea beetle damage during the two to three weeks following emergence can help to eliminate these problems (Sustainable Agriculture Facts, 1997).

Various beetles, including *Meligethes*, *Ceutorhynchus*, *Pylliodes* and *Phyllotreta* are the most important insect pests of oilseed rape in Europe and insecticidal control is imperative (Smith and Hewson, 1984; Hokkanen *et al.*, 1988; Cooper and Lane, 1991; Ekbom, 1991). For example, all oilseed rape fields in Finland are sprayed with insecticides at least once, usually twice to aid in the control of beetles (Hokkanen *et al.*, 1988; Hokkanen, 1989). Total management costs of beetle pests are estimated to be about one billion dollars worldwide (Palaniswamy, 1996). Despite a heavy reliance on chemical control of pests, it is estimated that 37% of all crop production worldwide is still destroyed by pests and diseases, with 13% of this attributed to insects (Gatehouse *et al.*, 1992).

1.2 The Flea Beetle (*Phyllotreta* Chevrolat)

Flea beetles are tiny insects, 2 to 3 millimeters long, which jump when disturbed, hence the name. Two main species constitute pests of canola. The crucifer flea beetle (*Phyllotreta cruciferae* Goeze), is black with a bluish sheen, and the striped flea beetle (*Phyllotreta striolata* F.), is black with two yellow stripes running the length of the body. Both species were introduced from Eurasia and are the most common and serious pests of canola. Flea beetles, known to be oligophagous herbivores, have a single generation per year in western Canada. The beetles overwinter as adults within leaf litter, grass, canola stubble and volunteer cruciferous plant debris, then emerge in April and early May once spring thaw begins. Warm, dry weather increases feeding activity and insect movement within the field. The flea beetle can completely destroy canola fields by feeding on cotyledons and young leaves. Damaged plants have a pitted or "shot-holed" appearance when the tissues around the feeding sites on the adaxial surface of the cotyledons die. Feeding may also occur on the petioles and stems (Burgess, 1977; Brandt and Lamb, 1994). Injury to cotyledons may result in delayed maturity, lowered yield and reduced seed quality. Heavy attacks can cause the seedlings to wither and die (Putnam, 1977; Lamb, 1984). Once the crop develops beyond the seedling stage, damage is minimal and the adult flea beetle population begins to decline.

Flea beetles customarily locate, attack and injure/destroy a crop quickly. Therefore control is difficult. Producers have a variety of cultural, biological and chemical controls available, however, no one method is totally effective. Studies conducted by Milbrath *et al.* (1995) and Alberta Agriculture (1999) have shown that the use of minimum tillage practices can help to reduce flea beetle populations. The pest prefers warm, dry soil conditions. When crop residue is buried, the soil readily absorbs the sun's rays and evaporation increases. This produces the flea beetles' preferred environment. Minimum tillage seeding may reduce flea beetle feeding and activity due to a cooler, dryer surface environment. Planting the crop later in the season showed significantly less flea beetle damage than earlier plantings (Alberta Agriculture, 1999). However, when flea beetle populations are high, there are no effective agronomic controls available.

Parasites, predators and diseases can aid in the regulation of flea beetle populations. Lacewing larvae (*Chrysopa carnea)*, big-eyed bugs (*Geocoris bullatus)*, the western damsel bug (*Nabis alternatus)* and the northern field cricket (*Gryllus pennsylvanicus)* are known to feed on flea beetles. The braconid wasp (*Microctonus vittatae)* parasitizes flea beetle adults but no overall effect on flea beetle numbers is known. Most flea beetle populations emerge in such large numbers and during such a short time period that most parasites and predators are overwhelmed and rendered ineffective (Sustainable Agriculture Facts, 1997).

Seed dressing insecticides based on Lindane such as Vitavax RS Flowable and Rovral ST provide limited control for seven to ten days after crop emergence. Granular insecticides placed in the soil, such as Furadan and Counter provide protection for up to

21 days following emergence but generally are not very effective at the onset of emergence. Unfortunately, both seed dressings and granular insecticides do not provide consistent and effective control during times of heavy infestation. Foliar application of various insecticides has been studied (Weiss *et al*, 1991). Although foliar applications based on the chemical carbaryl were effective, the cost and efficiency versus those of traditional seed treatments were discouraging factors. The development of resistance to insecticides is also a major problem with chemical controls. The most effective chemical control for flea beetles has been carbofuran. Studies have shown that flea beetles are developing resistances to this and other insecticides (Turnock and Turnbull, 1994).

Chemical control is expensive and raises legitimate environmental concerns. Carbofuran and Lindane are highly toxic chemicals that pose both human health and environmental hazards (Palaniswamy, 1996). Lindane (hexachlorocyclohexane) is a hydrophobic organochlorine that has a high affinity for lipids. Once a person is exposed to Lindane, the chemical is distributed to and stored in all cell organs and tissues in the human body (Shaw, 2000). Additionally it is a probable carcinogen and is very toxic in aquatic and soil environments. Canada and the United States have agreed to a voluntary total elimination of Lindane use in canola seed treatments by July 1, 2001 (Germination, January 1999) due to the environmental and human toxicity of the chemical.

Non chemical means of control have received attention in recent years. Work has been initiated attempting to increase insect resistance in the host species by changing the amount, type and structure of the leaf surface wax (Palaniswamy, 1996). Bodnaryk (1992b) found that flea beetles fed on waxy leaves at a lower rate than on non-waxy leaves and that the feeding pattern depended upon the leaf waxiness. Increased feeding was documented on the edges of waxy leaves and random feeding was seen on non-waxy leaves.

Cysteine and aspartic type proteinases have also been isolated from the gut of flea beetles and work has been initiated to develop transgenic canola, which produces proteinase inhibitors against various pests, including flea beetles (Rymerson and Bodnaryk, 1995). Zaplachinski (1999) documented an improvement in flea beetle resistance in transgenic canola seedlings through the expression of a cysteine proteinase inhibitor in the first 5 to 10 days after germination. Flea beetle trials showed that the transgenic lines containing the cysteine inhibitor were more resistant to flea beetle attack when compared to the control plants. However, issues are now being raised as to the suitability of transgenic crops for human consumption. Transgenic crops (genetically modified organisms) have come under a great deal of scrutiny and criticism and as a result have limited appeal and acceptance by consumers.

1.3 Host Plant Resistance

One of the major objectives of the plant breeder is to produce plants with durable pest resistance. The advantages of developing resistant crops as opposed to prolonged reliance on chemical controls are numerous. Crop resistance offers season long protection, independent of weather, and has no application costs. Insect pests are affected at the most sensitive stage and in many cases only the crop-eating insects are affected. Plant tissues, the environment and the consumer are all protected from excessive pesticide application (Gatehouse *et al.*, 1992). Producers can achieve an economic advantage with the use of resistant crops due to reduced chemical usage and additional crop harvested.

Host plant resistance to insect attack is based on one of three types of resistance mechanisms (Painter, 1951). These are non-preference or antixenosis (Kogan and Ortman, 1978), antibiosis, and tolerance (Palaniswamy, 1996). Antixenosis or non-preference is the result of plant characteristics that prevent, reduce or limit insects from approaching, landing, settling and feeding. This may be caused by chemical and/or physical deterrents. Plant characters that influence non-preference include color, light reflection, type of pubescence, leaf angle, odor and taste (Fehr, 1991). Antixenosis resistance can reduce the rate at which the insect population increases by reducing the initial attack population or limiting the size of the successive generation.

Antibiosis is the adverse effect of plant tissue used as food by an insect on the insect's development and reproduction (Fehr, 1991). These plant attributes include toxins and growth inhibitors. Antibiosis will reduce the rate at which the insect population increases by reducing reproduction and survival and increasing generation time (Kennedy *et al.*, 1987). Antibiosis is considered to be the only true form of insect resistance in plants (Fehr, 1991).

Tolerance is usually defined as the ability of the plant to endure the pest with little or no reaction, as shown by a complete absence of symptom expression and damage (Bos and Parlevliet, 1995). A tolerant and nontolerant cultivar would be indistinguishable when rated for the number of insect pests present, but the tolerant variety would be less affected by the pest (Fehr, 1991). The degree of a plant's tolerance is based on its capability to compensate for insect damage by being able to repair, grow and reproduce

(Palaniswamy, 1996). Tolerance raises the level of damage a crop can handle before its economic yield is adversely affected (Kennedy *et al.*, 1987).

The development of host plant resistance has limitations. Over the years, crop varieties with high and consistent yields, nutritional value and adaptation to the growing environment have been selected. Consequently, few cultivated species have retained the degree of pest resistance expressed in their wild relatives (Feeny, 1976; Gatehouse *et al.*, 1992). Pest resistances are among the traits found in related species and other plant crops when not present in economically important cultivated crops (Hansen and Earle, 1997).

Resistant germplasm is needed for the successful development of crop resistance. Variation in the response of other cruciferous plants to flea beetle attack has been studied (Brett and Rudder, 1966; Putnam, 1977, Lamb, 1984) and *Phyllotreta* species have shown differential feeding responses to various cruciferous crops. The use of other crops that exhibit insect resistance as a source of resistance does show promise as a method of introducing flea beetle resistance in commercial *B. napus* crops (Lamb, 1980; Meisner and Mitchell, 1983).

Varying levels of flea beetle tolerance and/or antixenosis have been identified in other species such as *Crambe abyssinica* Hochst, *Brassica juncea*, a Mediterranean weed *Brassica villosa* Biv., and *Sinapis alba* (Palaniswamy *et al.*, 1992; Palaniswamy and Bodnaryk, 1994; Brown *et al*, 1999). Accessions of *B. carinata*, *B. juncea* and *S. alba* that are 3 to 4 times more resistant to flea beetles than other accessions of the same species have been reported (Palaniswamy, 1996). Excluding cruciferous weeds, *S. alba* is the most resistant species for flea beetles, followed by *B. carinata*. Yellow mustard (*S. alba*) is phylogenetically closer to *Brassica* species than other resistant crops (Warwick and Black, 1991) and has ample chromosomal homoeology as indicated by both chromosomal pairing and chromosomal synteny (Lelivelt *et al.*, 1993). The availability of highly resistant and susceptible accessions of the same species provides an opportunity to study the chemical and morphological causes of insect resistance, to develop genetic markers for resistance and to identify resistance genes (Palaniswamy, 1996).

Developing an insect resistant cultivar using traditional plant breeding methods can take years. Generally, resistance to insect pests is partial and polygenically controlled (Palaniswamy, 1996) and therefore difficult to isolate. Desired genes can be transferred to recipient plants however, at the same time there can be a co-transfer of undesirable characteristics. Plant resistance to one pest may increase susceptibility to another pest. This becomes a significant problem in crops where several pests need to be controlled concurrently. Resistance based on plant-based chemical and physical deterrents may have injurious effects on natural enemies of the insect pest (Herzog and Funderburk, 1985). A single gene change altering the production of one different metabolic enzyme may result in the production of a multitude of new secondary compounds which may have deleterious effects within the crop or impact the crop's marketability (Kennedy *et al.*, 1987). As a result, plant breeders have not had great successes in the quest to develop insect resistant varieties.

Evaluation of oilseed rape varieties for flea beetle resistance is labor intensive and to date no host plant resistance for flea beetle has been identified in commercial *Brassica napus* cultivars (Brandt and Lamb, 1994). Field evaluation often results in high variability due to weather effects on crop growth, beetle feeding and beetle density (Palaniswamy and Lamb, 1992). The experimental screening period is limited to a short time in the spring when flea beetles are the most active (Lamb, 1988). Field screening methods based on beetle counts and measurements of plant growth, plant survival, and plant damage (Brett and Sullivan, 1974; Lamb, 1988) have been developed but do not overcome all problems of field evaluation techniques. The development of resistant cultivars is made more difficult by the fact that *Phyllotreta* species are difficult to rear in the laboratory environment. Alternately, in small scale field trails the flea beetle feeding damage can be so devastating that even large differences among cultivars are difficult to detect (Lamb, 1988).

1.4 *Sinapis alba*

Sinapis alba L. is grown in Europe and Canada predominately as a condiment crop. It is tolerant to high temperatures, withstands drought stress (Downey *et al*., 1975), is shatter resistant (Brown *et al*., 1997) and has a high seed yield (Gareau *et al*., 1990). As an oilseed crop it offers poor oil quality and intermediate erucic acid levels and is therefore limited for use as a source of seed meal for livestock feed.

Sinapis alba is a valuable source for introgression of desirable traits into *B. napus* and has a promising potential as a source for insect resistance for canola. *Sinapis alba* L. is a diploid member of the *Brassiceae* tribe (2n=24, SaSa genome) (Hemmingway, 1976) and can cross with other *Brassica* species. Hybrids are difficult to generate through traditional plant breeding methods due to recombination difficulties between the *Brassica* and *Sinapis* genomes (Salisbury and Kadkol, 1989). Recently, sexual hybridizations between *S. alba* and *B. napus* have been successfully achieved through the use of embryo rescue techniques (Ripley and Arnison, 1990; Chevre *et al*., 1994). Characteristics from both parents were equally expressed in intergeneric hybrids. This is an advantage since it allows for selection for specific characters (oil composition, glucosinolate levels) within hybrid progeny and may allow introgression of desirable characteristics of *S. alba* into the *B. napus* genome via backcrossing (Brown *et al.*, 1997)

Sinapis alba shows a high level of resistance in the field to damage from adults of *Phyllotreta* species (Putnam, 1977; Lamb, 1984). The cotyledons of *S. alba* have multiple resistance mechanisms, including tolerance (Brandt and Lamb, 1994), constitutive antixenosis (Bodnaryk and Lamb, 1991), and wound-inducible resistance (Bodnaryk, 1992a; Palaniswamy and Lamb, 1993). Various studies have attempted to determine the mechanisms of the flea beetle resistance in *S. alba*. The role of trichomes and leaf waxiness was studied in conjunction with resistance to flea beetle feeding. It was observed that stiff hairs on the pods of *S. alba* protect the pod from feeding damage in later stages of the crop development (Lamb, 1980). In another study it was found that flea beetles fed on waxy leaves at a lower rate than on non-waxy leaves and the feeding pattern differed depending on the degree of leaf waxiness (Bodnaryk, 1992b).

Glucosinolates (sulfer-containing glycosides found in all members of the cruciferae) and their breakdown products may protect plants from some generalist pests and pathogens (Giamoustaris and Mithen, 1995). These compounds are reported to have different effects depending upon their type and concentration. There has been extensive work in studying the role of specific glucosinolates in the flea beetle resistance. Six glucosinolates have been detected in tissues of *S. alba*, including benzyl, *p*-hydroxybenzyl (sinalbin), 3-indolyl methyl, isobutyl, 4-hydroxy-3-indol methyl, and phenylethyl glucosinolate. Sinalbin is the dominant glucosinolate, accounting for over

90% of the glucosinolates found in the cotyledons (Hopkins *et al.*, 1998) and has been implicated in resistance to flea beetle, bertha armyworm (Bodnaryk, 1991) and *Lygus* bugs (Bodnaryk, 1997). *Brassica napus* seedlings do not produce this form of glucosinolate (Bodnaryk, 1991; Brown *et al.*, 1997). However, in several studies, lines of *S. alba* differing by 1000-fold in their sinalbin concentration showed the same level of antixenosis against flea beetles, suggesting that resistance may be independent of glucosinolates (Bodnaryk, 1991, 1992a, 1997). In another study, artificial cotyledon wounding did not significantly alter levels of various glucosinolates. No correlation could be found between wound- induced resistance, and the presence, absence or change in any glucosinolate in the cotyledons of *S. alba* (Bodnaryk, 1992a).

Moderate concentrations of benzyl glucosinolate (glucotropaeolin) have been found in *S. alba* tissues (McCloskey and Isman, 1993; Hopkins *et al.*, 1998). Benzyl isothiocyanate, a breakdown product of glucotropaeolin, has been shown to be toxic to some insect pests (Bartelt and Mikolajczak, 1989). However, intact glucotropaeolin stimulates flea beetle feeding (Nielsen, 1978) and oviposition of *Pieris rapae* L. (Huang and Renwick, 1994), so it is unclear as to its role in *S. alba* flea beetle resistance.

Studies on insects other than flea beetles indicate no consistent relationship between glucosinolates and crop resistance (Åhman, 1982; Rawlinson and Williams, 1991). This is particularly true in Canada and Europe where the majority of crop area seeded has shifted from cultivars of high seed glucosinolate levels to cultivars of low seed glucosinolate levels and no significant changes in the levels of insect pest damage have been observed (Åhman, 1982; Lamb, 1989).

1.5 Molecular Approaches

Genetic enhancement from sources other than cultivated forms involves the exploitation of germplasm from wild, weedy species or landraces to introduce new agronomic characters. The value of wild relatives as sources of new germplasm is well established in breeding programs for various crops. However, the efficiency with which wild germplasm is utilized for introducing pest resistance and other agronomic characters into elite cultivars varies greatly (Lagudah and Appels, 1994). A common problem using alien species in crop improvement is the introduction of undesirable genetic material accompanying the target gene being introgressed.

Cultivated tomato (*Lycopersicon esculentum*) crops are attacked by a series of insect pests and root knot nematodes causing major economic damage. One of these microscopic organisms, *Meloidogyne incognita,* is an obligate endoparasite that penetrates plant roots near the tip and migrates intercellularly to a feeding site in the developing vascular cylinder (Williamson and Hussey, 1996). Here, the nematode initiates formation of galls (or root knots) and specialized feeder cells in the host from which they derive nutrients. Cytoplasmic fluids are removed from the host, which greatly affects nutrient partitioning and water uptake. Symptoms include poor fruit yield, stunted growth, wilting and susceptibility to other phytopathogenic viruses. In order to control the nematode, a defense mechanism that blocks ingestion of nutrients by inducing physical or chemical changes in penetrated cells or sieve elements is required (Rossi *et al.*, 1998).

No resistance has been achieved through traditional plant breeding methods however; a single, dominant gene (*Mi)* that mediates resistance against three major

Meloidogyne species was found in a wild relative, *L. peruvianum* and introduced into cultivated tomato by embryo rescue (Smith, 1944). The *Mi* gene was isolated from a *L. peruvianum* population segregating for resistance by positional cloning using PCR, RFLP and AFLP markers (Kaloshian *et al.*, 1998; Milligan *et al.*, 1998). The gene was localized to a small region of tomato chromosome 6 by identification of recombinants, and DNA sequence analysis was carried out to identify *Mi* candidates. Sequencing revealed two genes *Mi*-1.1 and *Mi*-1.2, which were 95% identical to each other and encoded proteins with high similarity to previously cloned plant resistance genes. Highest similarity is to the tomato gene *Prf,* which together with *Pto*, is required for resistance to *P. syringae* carrying the avirulence gene *avrPto (*Salmeron *et al.*, 1996). With the assistance of linked markers, such as the isozyme marker *Aps-1* and the DNA marker *Rex-1*, *Mi* has been incorporated into many modern tomato cultivars (Medina-Filho and Tanksley, 1983; Williamson *et al.*, 1994).

The Hessian fly *Mayetiola destructor* is one of the most destructive pests of wheat crops. Resistant cultivars are available and there is a gene-for-gene relationship between resistance genes in wheat and the avirulence of the Hessian fly biotypes that determines the evolution of the fly (Hachett and Gallun, 1970). Most resistance genes are dominant or partially dominant, and cause antibiosis in the fly larvae. However, deployed genes are periodically overcome by new virulent biotypes so identification of new resistance genes is required.

For over 40 years, the wild grass *Aegilops ventricosa* has been a source of important genes that govern characteristics of agronomic interest for wheat breeders. *Aegilops ventricosa* is an allotetraploid ($D^vD^vM^vM^v$) and is partially homologous to

Triticum aestivum (AABBDD). Resistance to *M. destructor* has been observed in several accessions of *Ae. ventricosa* (Gill *et al.*, 1985; Amri *et al.*, 1992). A population of 70 plants was derived from crossing *T. turgidum* x *Ae. ventricosa* x *T. aestivum*. One line (H-93-33) showed little or no infestation by the Hessain fly. Using cytological methods and RFLP/isozyme markers, H-93-33 was characterized as carrying chromosome $4M^v$ from *Ae. ventricosa*. Delibes *et al.* (1997) reported this inheritance of Hessian fly resistance as a single Mendelian factor (H27) and that this transferred gene is linked to a phosphatase isozyme marker (Acph-M^v1) from the $4M^v$ chromosome. The H27 gene was the first to be mapped on an M-genome chromosome that has been introduced into wheat and could provide a broader range of genetic resistance for the Hessian fly.

Plant genetic manipulation can make a significant contribution in the production of insect-resistant crops. As opposed to conventional plant breeding methods desired gene(s) can be transferred to the recipient plants without the co-transfer of undesirable traits using molecular approaches. This can greatly "speed up" the development of new crop varieties. Additionally, the transfer of genes can be achieved across incompatibility barriers so resistance genes can be introduced from sources that are unavailable through traditional breeding methods (Gatehouse *et al.*, 1992).

One method of molecular analysis of genes involved in important developmental processes in plants is the identification of DNA sequences closely linked to the target trait. Molecular genetic markers permit a detailed analysis of plant genomes by allowing the construction of linkage maps, which provide starting points for studying specific regions of the genome (Lagudah and Appels, 1994). Linkage maps have been constructed for *B. juncea* (Cheung *et al.*, 1997), *B. napus* (Sharpe *et al.*, 1995; Parkin *et* *al*., 1995), wheat (Chao *et al*., 1989), barley (Blake *et al*., 1989), maize, tomato and lettuce (O'Brien, 1990). Linkage maps have expanded our ability to study genetic diversity, genome organization and genome relationships (Chang and Meyerowitz, 1991).

The applications of molecular markers in plant-breeding programs are varied. Molecular markers can trace agronomic characters that are difficult or expensive to measure, increase the efficiency of backcrossing and verify the existence of a true F_1 hybrid from a difficult cross. Markers can be used to assess germplasm diversity, trace resistance genes that are sporadic and identify varieties for plant variety registration purposes (Lagudah and Appels, 1994). Molecular markers using protein profiles or isozymes permit the early screening of plants for the trait of interest, and are not influenced by environmental factors (Chang and Meyerowitz, 1991). Genetic markers can be provided for the pyramiding of pest resistance genes and to characterize pathogen populations.

Genetic mapping of insect resistance requires reliable polymorphic genetic markers in a well-defined segregating population. Molecular markers can be used to identify individual progeny that have received only the desirable resistance gene(s) and may provide a starting point for the cloning of the gene(s). Techniques frequently used in identifying molecular markers defining DNA target sequences are DNA hybridization and Restriction Fragment Length Polymorphism (RFLP). RFLPs obtained from sequence variation are revealed in DNA hybridization as changes in the length of DNA fragments after cleavage by restriction enzymes (Lagudah and Appels, 1994). Restriction fragment length polymorphism-based markers have been reported in plants, animals and human genetic studies and used in the construction of genetic maps. Restriction fragment length polymorphism markers have been used in the identification of mono-and polygenic loci controlling disease and other agronomic traits. In many crops, RFLP markers target introgressed regions carrying viral, bacterial, fungal and insect resistance genes and these markers are being used in breeding programs. Genotypes derived from crossing programs can be identified using RFLP markers and allow individuals whose genome composition most resembles the recurrent parent to be selected for the next cross. In this manner, RFLP markers allow the acceleration of the introgression of traits from genetically distant germplasm sources (Young and Tanksley, 1989).

1.6 Quality Components

The Canadian Food Inspection Agency, Plant Health and Production division sets seed quality parameters for new commercial canola cultivars that must be met prior to registration. For the year 2001, the canola quality definition will require that the seeds of newly registered cultivars have a total glucosinolate content of <18 µm/g oil free meal and < 1% erucic acid, (Canola Council of Canada, 2000).

1.6.1 Glucosinolates

Glucosinolates are sulphur-containing glycosides found within *Brassica* and relatives. Structurally, the glucosinolate molecule has two parts; a glycone moiety and a variable side chain. Glucosinolates in *Brassica* can be divided into three major classes on the basis of the structure of the side chain, which is derived from α-amino acids, (Magrath *et al.*, 1993). The three groups are; (1) aliphatics/alkenyl derived from methionine, (2) indolyls derived from tryptophan and (3) aromatic/aralkyls derived from phenylalanine. Of the three groups, the aliphatic series is the most abundant. Aliphatic glucosinolates are thioglycosides that occur in the leaves of the plant and *B. napus* has a restricted and uniform aliphatic glucosinolate profile. It contains butenyl and pentenyl glucosinolates and their hydroxylated analogues, with trace levels of other glucosinolates occasionally reported (Magrath *et al.*, 1993).

The aliphatic glucosinolate hydrolysis pathway is as follows:

Methionine → Aliphatic Glucosinolates → $\underline{\text{Myrosinase}}$ →Isothiocyanates

(Vaughan *et al.*, 1976; Fenwick *et al.*, 1983; Larsen *et al.*, 1984). The enzyme myrosinase is released upon tissue damage, and hydrolyzes aliphatic glucosinolates to produce isothiocyanates. Isothiocyanates are responsible for the flavor of *Brassica* and other cruciferous crops and the level within seeds influences the quality of the meal following oil extraction (Mithen *et al.*, 1987; Bartlet *et al.*, 1992). These compounds have been shown to attract specialized cruciferous pests and repel generalist herbivores such as slugs and birds.

The inheritance of glucosinolate biosynthesis is complex and is regulated by alleles at several loci that determine the total amount of glucosinolates and side-chain structure (Magrath *et al.*, 1993). Genetic modification of total aliphatic levels in *B. napus* seed has not resulted in a change in the ratio of individual aliphatic glucosinolates or in the level and ratio of individual indolyl glucosinolates within vegetative tissues. This is evidenced by the fact that levels of residual aliphatic glucosinolates in leaves of agronomically superior low glucosinolate cultivars are similar to those leaves from previously grown high glucosinolate cultivars (Milford *et al.*, 1989; Porter *et al.* 1990; Inglis *et al.*, 1992).

Three major glucosinolate components are found in *S. alba* seeds and seedlings, sinalbin (4-hydroxybenzyl glucosinolate), 4 hydroxy-benzylamine and ϒ-glutamyl-4-hydroxybenzylamine (Larsen *et al.*, 1984). Although these are metabolically related, 4 hydroxy-benzylamine and ϒ-glutamyl-4-hydroxybenzylamine are not present in *B. napus* (Horn and Vaughn, 1983). There is evidence that *Brassicaceae* insect pests may be more responsive to individual glucosinolate components in the vegetative tissues as opposed to total glucosinolates. Therefore the composition and ratio of plant glucosinolate components may be a more important stimulant than total glucosinolate concentration for controlling plant-insect pest interactions (Giamoustaris and Mithin, 1995).

1.6.2 Seed Oil and Protein

Sucrose translocated to the seed during seed development is metabolized to precursors for protein and oil, the major components of mature rapeseed. It has been well established that protein and oil content in the seed are negatively correlated and inversely influenced by the environment (Johnson *et al.*, 1955; Kwon and Torrie, 1964; Shannon *et al.*, 1972.) Despite this, plant breeding programs have shown that positive advances for both seed protein and oil levels can be made simultaneously by selection for the sum of these two traits. It is generally accepted that the genetic control of oil and protein is under additive genetic control with slight dominance traits (Stefansson and Kondra, 1975; Grami and Stefansson, 1977).

Brassica seed oils are composed of storage triacylglycerols that are synthesized by the endoplasmic reticulum from fatty acid acyl chains. The fatty acid chains are generated by the plastid in the developing seed (Hills *et al.*, 1995). The specific fatty acid components and their levels determine the quality and composition of the oil. *Sinapis alba* seed oil is valuable for industrial purposes only, due to a high concentration of erucic acid (Yaniv *et al*., 1998). A more detailed look at the fatty acid components and their relationships with seed, plant tissues and each other follows in Section 1.6.3 and 1.6.4.

1.6.3 Seed Fatty Acid

Triacylglycerols constitute a major source of edible fat (Ohlson, 1972) and compositional changes in lipids and their constituent fatty acids during seed maturation have been studied in *B. napus* (Sims, 1964; McKillican, 1966), *C. abyssinica* (Sims, 1964; McKillican, 1966; Gurr *et al*., 1972) and *S. alba* (Dasgupta and Friend, 1973). Long chain monounsaturated fatty acids such as gadoleic and erucic acids are the major components of seed triacylglycerols in cruciferous crops that are of interest for the chemical industry.

The pathway for synthesis of the major fatty acids is as follows:

$$\begin{tikzcd}
C_2 \text{ pools} \arrow[r] & \text{Palmitic acid (C16:0)} \arrow[r] & \text{Stearic acid (C18:0)} \arrow[d] \\
\text{Erucic acid (C22:1)} & \text{Gadoleic acid (C20:1)} \arrow[l] & \text{Oleic acid (C18:1)} \arrow[l] \arrow[d] \\
 & \text{Linolenic acid (C18:3)} & \text{Linoleic acid (C18:2)} \arrow[l]
\end{tikzcd}$$

(Downey, 1990).

In the majority of cruciferous crops, seed maturation and lipid accumulation begin with a sharp decline in the levels of palmitic, linoleic and linolenic acids, however the decrease in linolenic acid is less dramatic in *S. alba* than in *C. abyssinica* (Gurr *et al.*, 1972). *Sinapis alba* also shows evidence of a rapid accumulation of oleic acid for the first 4 weeks of seed maturation but then accumulation plateaus and remains unchanged (Mukherjee and Kiewitt, 1998). Accumulations of gadoleic acid, erucic acid are seen during this period and are consistent with an elongation of oleic acid to the long-chain monounsaturated fatty acids (Downey and Craig, 1964; Appleby *et al.*, 1974; Pollard and Stumpf, 1980).

Liu and Brown (1996) and Yaniv *et al.* (1998) found that the fatty acid composition in the cotyledon tissue of oilseeds during seed germination changed only slightly. However, the fatty acid composition of newly differentiated tissues was significantly different from that of the original seed, specifically higher in palmitic and linolenic acids and lower in oleic acid. It was also noted that the fatty acid composition of each new tissue was highly conserved, regardless of the genotype of the original seeds. Conserved levels of fatty acids in cotyledons indicate that storage lipids in the cotyledons may be broken down and utilized on the basis of individual and specific triacylglycerols rather than total fatty acids. In *S. alba* and *C. abyssinica*, the utilization of the storage lipids during germination is accompanied by a decrease in erucic acid in the cotyledons and a disappearance from the roots (Yaniv *et al.*, 1998). The different fatty acid profiles seen in seeds versus tissues formed after germination may provide information with respect to insect feeding patterns and host preference. Of particular interest for flea beetle studies may be the changes seen in the fatty acid levels in cotyledons of cruciferous plants.

1.6.4 Half Seed Fatty Acid Determination

The half-seed analysis method, proposed by Downey and Harvey (1963), has been one of the most powerful methods used by plant breeders. The half seed technique allows breeders to ascertain the fatty acid composition of a single seed that can still be grown to maturity. This methodology has been used to select canola cultivars for oil with fatty acid compositions that meet market demands such as low erucic acid or high oleic acid content (Yermanos, 1968; Roy and Tarr, 1987). It has also been used in sunflower (Conte *et al.*, 1989) and pea breeding programs (Swiecicki *et al.*, 1982). The half-seed method allows for a rapid and economically efficient evaluation of breeding lines. The technique entails the separation of the one cotyledon from the rest of the seed. The cotyledon is analyzed; the remaining seed is grown to a mature plant. The half-seed permits the characterization of the seed that will eventually be harvested from the individual plant. Studies of half seed fatty acid properties of cotyledons may provide information on flea beetle feeding patterns.

1.7 Focus and Objectives of This Research

The focus of this research was to identify genetic sources of flea beetle resistance within a hybrid *B. napus/S. alba* population and determine the genetic basis of this resistance using molecular markers. Specifically, a double haploid (DH) population of 251 *B. napus* plants, that segregated for flea beetle resistance, was developed from a cross between a resistant hybrid and susceptible *B. napus*. Eighty individuals from the population were selected for further testing and constituted the final mapping population. Seventy-eight genomic and cDNA clones were used as RFLP markers in the development of an RFLP linkage map for this population. Flea beetle damage rating and seed quality analysis (oil, defatted protein, glucosinolate and fatty acid levels) were determined for the parental lines and the DH population.

II. Materials and Methods

2.1 Development of the Mapping Population

Double haploid lines were developed from interspecific crosses (*Sinapis alba* x *Brassica napus* backcrossed to *Brassica napus* three times) performed at the Univeristy of Guelph between three *B. napus* cultivars, Topas, Westar, CrGC5006, and three *S. alba* cultivars, Kirby, Emergo, PGR13245 (Ripley and Arnison, 1990). Three hundred ten DH lines were characterized for their flea beetle resistance in multi-year testing at the University of Alberta (Appendix 1). Two lines (designated as LK-001 and LK-569) showed reliable flea beetle resistance and were selected as resistant parents. A susceptible *B. napus* DH line 94-99 (University of Alberta) was selected as the other parent due to its high microspore embryogenic response, and superior agronomic traits.

In cross #1, several LK-569 plants were used as the paternal parent and several 94-99 plants were used as the maternal parent used to create F_1 progeny seed. The reciprocal cross was also done, where 94-99 was the paternal parent and LK-569 was the maternal parent. Cross #2 used several LK-001 plants as the paternal parent and several 94-99 plants as the maternal parent to create F_1 progeny seed. The reciprocal cross was also completed with 94-99 as the paternal parent and LK-001 as the maternal parent. The F_1 progeny seed for each individual cross was harvested and bulked. The bulk F1 progeny seed from the LK-569 x 94-99 cross and the bulk F1 progeny seed from the LK-0019 x 94-99 cross underwent microspore culture (Chuong and Beversdorf, 1985) to create two separate populations (Figure 1). Microspore culture of the two populations was conducted by Mohan Thiagarajah in the Department of Agricultural, Food, and

Nutritional Sciences, University of Alberta. The resulting microspore derived plants were exposed to 0.33 % colchicine for 2 hours, 15 minutes to promote chromosome doubling and once flowering began, underwent self-pollination. The population derived from the LK-569 x 94-99 cross was designated as 98-A-4 and consisted of 222 F_2DH *B. napus* plants. The population derived from the LK-001 x 94-99 cross was designated as 98-B-4 and consisted of 251 F_2DH *B. napus* plants. Of these two populations, based on seed quantity, 80 individuals from the 98-B-4 population were selected for the mapping population.

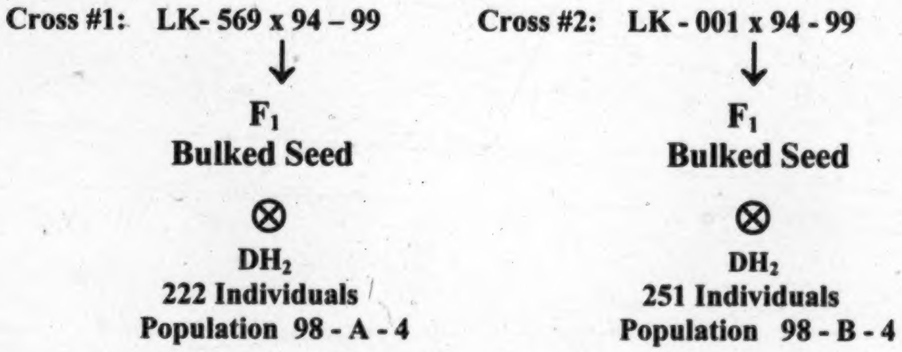

Cross #1: LK- 569 x 94 – 99
F_1
Bulked Seed
⊗
DH_2
222 Individuals
Population 98 - A - 4
Cross #2: LK - 001 x 94 - 99
F_1
Bulked Seed
⊗
DH_2
251 Individuals
Population 98 - B - 4

Figure 1: Development of the DH populations segregating for flea beetle resistance.

2.2 Screening for Antixenosis Resistance to Flea Beetles

Flea beetle feeding trials were performed at the University of Alberta with the help of Rebecka Carroll and Lesley Davis. Screening and data analysis for antixenosis resistance to flea beetles was essentially conducted as described by Gavloski *et al.* (2000). Adult crucifer flea beetles (*P. cruciferae)* were collected from field populations from May to August near Vegreville and Edmonton, Alberta, using allylisothiocyanate-baited traps (Figure 2); (Burgess and Wiens, 1980), or a vacuum insect sampler. Beetles

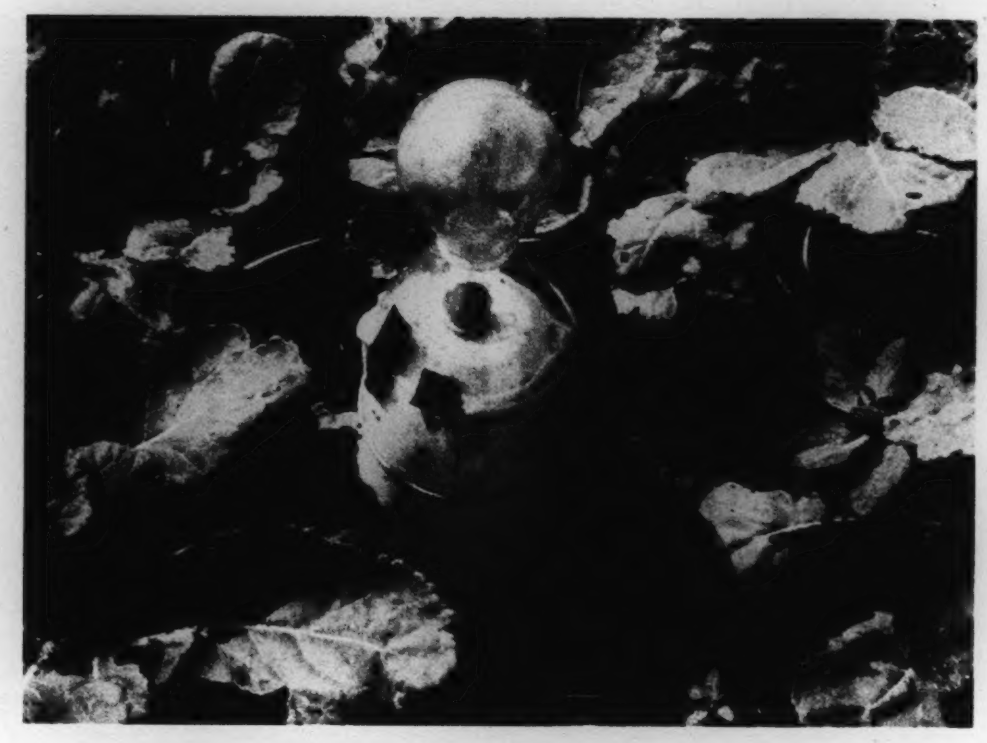


Figure 2: Allylisothiocyanate-baited traps for flea beetle collection (Burgess and Wiens, 1980).

were held in screen cages in a room at 23^{0}C with a 16L: 8D photoperiod and fed canola and cabbage leaves.

Seedlings were grown individually in a growth chamber at 15^{0}C (day) and 12^{0}C (night) in 3 cm x 5 cm round plastic vials filled with Metro-Mix 220 (Apache Seeds, Edmonton, Alberta). The bottom of each vial was punctured to facilitate watering and were held in greenhouse flats and watered by sub-irrigation. Seven-day-old seedlings (cotyledon stage) were selected for uniformity and used in the screening experiments.

Screening was conducted in Plexiglass arenas with nylon-screen tops, essentially as described by Palaniswamy *et al.* (1992). Arenas were 35-cm tall and 43 x 43 cm square, with a plastic foam base (43 x 43 cm square and 2.5 cm thick) supported by a Plexiglass ridge about 6 cm from the bottom of the arena. The base had 100 holes (3 cm diameter) evenly spaced in a 10 x 10 layout with a spacing of 0.8 cm between holes. Vials containing seedlings were inserted into the holes so that vial rims were even with the foam base surface. Holes were numbered sequentially from 0-99, producing a 10 x 10 layout or 10 rows and 10 columns. Ten plants of 10 lines were placed in the arena as a 10 by 10-Latin square design (each entry appeared once in each row and each column in random order). *Brassica napus* cv. Quantum was used in each test as a standard against which the level of resistance for the other nine lines was compared. This enabled data screened in different arenas to be compared. Quantum was selected to be the standard as it is a commonly grown commercial canola cultivar. For ease of identification, a small marker was placed in the vials containing Quantum. Approximately 500 beetles were introduced to the arena through an opening on the front of the arena, and the opening was then sealed with a plastic cover. The beetles were distributed as evenly as possible through out the arena so to assure all seedlings were exposed to flea beetle attack. Additional beetles were added as needed to maintain populations near 500 throughout the length of the screening experiment.

Feeding damage to each cotyledon was rated visually using a scale from 0 (no damage) to 10 (cotyledon destroyed); thus, a rating of 1 was assigned if approximately 10% of the area of the cotyledon was damaged. Each cotyledon was rated separately and the values of the two cotyledons were summed and multiplied by 5 to estimate the percent damage of the foliage area of the whole seedling. The beetles sometimes fed on petioles and stems, which caused death of cotyledons even if the cotyledon surface had not been attacked. Regardless of the amount of damage to the cotyledon, a rating of 10 was assigned when the petiole was cut and the cotyledon was severed or wilted beyond recovery. Both cotyledons were given a rating of 10 when the stem was cut and the seedling felled. The amount of damage to Quantum was examined regularly. Once damage to Quantum was approximately 50%, damage ratings were taken for all plants and the test ended. All tests were conducted in a room at 23^0C±1 with a light cycle of 16L: 8D.

2.3 Analysis of Flea Beetle Damage Data

Data analysis was as described in Gavloski *et al.* (2000). In order to make the tests uniform and comparable between arenas and separate screening sessions, the score for every individual line was corrected relative to Quantum damage at 50% using the following equation:

CS = DM x 0.5/QD x100

where CS = corrected score

DM = mean of % damage of individual line

QD = mean damage of Quantum in %

Each group of 10 lines was tested in two arenas, resulting in 20 replications per line. The 98-B-4 population was tested twice under the same conditions resulting in 40 replications per line. Data was analyzed as a Latin square design using analysis of variance (SAS Institute, 1985). Duncan's new multiple range test ($P<0.05$) was used to separate the means. Analysis was run for the pooled data of the two arenas, and also separately by arena. Results presented within this thesis are from pooled data.

2.4 Genomic DNA Extraction

Information for solutions and buffers is provided in Appendix 7. Genomic DNA extraction was conducted essentially as described by Sharpe *et al*. (1995). Approximately 0.5g of freeze-dried tissue was milled to a powder for 1 hour. The powder was transferred to 15 mls of Kirby mix in 50ml polypropylene centrifuge tubes and tubes agitated for 15 minutes. The tubes were further agitated for 15 minutes on addition of 15 mls of a phenol/chloroform mix. The tubes were then centrifuged at 3000 rotations per minute (rpm) for 20 minutes at room temperature. The upper layer of supernatant was then removed to fresh 50 ml tubes. Approximately 0.1 volume of 3M sodium acetate and 0.6 volume of isopropanol were then added to the supernatant in fresh 50 ml tubes. This was mixed and left to precipitate in the dark overnight.

The tubes were centrifuged (3000 rpm) and the pellet air dried for at least 30 minutes. The pellet was resuspended in 2mls 1x TE and 4 μg per ml RNAase by agitation at 37^{0}C. Two mls of phenol/chloroform were added to the resuspended pellet and centrifuged. The supernatant was then precipitated overnight in sodium acetate/isopropanol. The precipitate was again centrifuged and the pellet air-dried overnight before being resuspended in 0.3ml of 1x TE at 37^{0}C. The DNA preparation was finally stored in eppendorf tubes at -20^{0}C.

2.5 DNA Quantification

DNA samples were quantified using a fluorometric assay that incorporated the Hoescht reagent (DNA/Hoescht dye complexes fluoresce at 458nm when excited by ultra violet (U.V.) light at a 365nm wavelength) essentially as described by Ausubel (1989). Ten microlitres (µl) of each DNA sample were digested with 0.8 µl EcoRI (75 units per µl), 3µl 10x the appropriate restriction buffer and 16.2 µl sterile water for a minimum of four hours at 37^{0}C. Two µl of each digested DNA preparation were added to 2mls of 0.1µg per ml Hoescht 33258 (Sigma) in 1x TEN, mixed and a fluorometric measurement taken. Calculations were made from fluorometric readings of standard digested DNA samples of known concentrations. Small quantities (5µg) of the remaining digested DNA were viewed on a 0.8% agarose gel after staining with ethidium bromide to examine the quality of the DNA.

2.6 Southern Blotting and Hybridization

Southern blotting and hybridization were conducted as described by Sharpe *et al.* (1995) with minor changes. Thirty μg of each DNA preparation were digested overnight at 37^{0}C with two units of EcoRI per μg of DNA and 3 μl of 10x H buffer. The digested DNA samples were loaded on a 0.8% agarose gel in 1 x Tris Acetate buffer and run overnight at 40 volts. The gel was agitated for 15 minutes at room temperature in 500 ml of 0.25 M HCl solution and for 30 minutes in 500 ml of 0.4 M NaOH. The gel was blotted and DNA transferred to Hybond N$^+$(Amersham Pharmacia Biotech) by overnight alkaline transfer. After blotting, filters were washed in 2 X SSC solution and stored at room temperature in 2 X SSC.

Filters were soaked in 2 X SSC, and then rolled into a hybridization tube. Fifteen ml of pre-hybridization solution was added and the tubes were placed in the hybridization oven to rotate for 2 hr at 65^{0}C. The following reaction was set up; 1.2 μl of primer d(N)$_6$ (50 ng per μ1) and 2 μl of the PCR amplified insert (100-200 ng) of each RFLP probe, were denatured in a boiling waterbath for 5-10 minutes. The denatured probes were cooled on ice for 5 minutes and then 2 μl 10x dCTP oligo-labelling buffer, 0.8 μl DNA polymerase I Klenow fragment, 9.3 μl sterile water and 5.0 μl ^{32}P dCTP redivue (3000 Ci per mmol) were added (Feinberg & Vogelstein, 1983). The reaction was incubated for 2 hr at room temperature and stopped by adding 30 μl 80 mM EDTA. The resulting solution was centrifuged at 2000 rpm for 2 minutes at room temperature through a 0.5 ml Sepharose Cl-6B in 1XTE spin column. Herring Testes DNA (250 μl of 1 mg per ml) was added to the probe and denatured as before. The hybridization solution (4.75 ml) was added to each hybridization tube to be followed by the denatured probe. Filters were left in the oven to hybridize overnight at 65^{0}C.

Probes were washed twice from the tubes with 30 ml of wash A (2 X SSC, 0.1% SDS) for 15 minutes each. Filters were then removed and washed twice with 500 ml of wash B (0.2 X SSC, 0.1% SDS) for 30 minutes each at 65^{0}C. Each filter was wrapped in clingfilm and exposed to X-ray film (Kodak X-Omat AR) at -80^{0}C for 1-14 days, depending on the signal strength.

Probes were stripped off the filters by pouring on boiling Strip A (0.2 X SSC, 0.1% SDS) and gently shaking the filters in a box for approximately ten minutes. This was repeated two or three times to ensure the complete removal of the probe. The filters could then be used in another hybridization or stored in the fridge.

2.7 RFLP Probes

Tom Osborn, Univeristy of Wisconsin, provided all genomic or cDNA RFLP probes. In order to determine the potential level of polymorphism within the mapping population, all cultivars (Topas, Westar, CrGC5006, Kirby, Emergo, PGR13245, Delta) used in creating the parental lines were digested with EcoRI and parental screening filters were produced. Thirty-two genomic or cDNA clones were used to probe the parental background. Screening filters were prepared for the mapping population using genomic DNA from both mapping population parental lines and seven randomly chosen individuals from population 98-B-4. Genomic DNA of these lines was digested with the enzymes EcoRI, EcoRV, HindIII, XbaI, BamH and RsaI. Twenty-five probes including those that produced evidence of polymorphism from the first round of parental probing were screened with these filters. An additional set of smaller filters was produced using the identical parental genomic DNA as before and four individuals from the mapping population digested only with EcoRI. From previous probings, it was evident that the digestions with EcoRI alone provided a high degree of polymorphism within the population. Thirty-two additional probes were screened with these smaller filters. Once the mapping population filters prepared, using genomic DNA digested with EcoRI were ready, probes were chosen based on previous evidence of polymorphism seen in the earlier probings of the parental and screening filters. Seventy-eight genomic or cDNA clones, provided by the University of Wisconsin, were used to probe the 2 parental lines and 80 individuals of the 98-B-4 mapping population.

2.8 Linkage Analysis and RFLP Map Generation

Assignment of RFLP loci to the 18 linkage groups was done both with MAPMAKER/EXP version 3.0b (Lander *et al.*, 1987) and manually. The probability of markers being linked is given by the logarithm of odds score (LOD). Minimum LOD scores of 3.0 were used to initially assign loci to linkage groups, and three point and multipoint analyses were used to determine the most likely map position within the linkage group. Manual fine adjustments were done to minimize double crossovers within short map intervals. Double crossovers should be rare over short chromosomal distances due to individuals of the 98-B-4 population having been derived from a single round of meiosis from a single F_1 source plant.

2.9 Quality Analysis

The 80 lines of the 98-B-4 population were seed increased in the summer of 1999 near Didsbury, Alberta by Agricore, an agricultural seed company. The harvested seed was cleaned, catalogued and sub sampled for quality analysis. Three lines, 98-B-4-012, 102 and 177 were not harvested and seed was not submitted for quality analysis. All quality analysis was conducted at the University of Alberta by Ann Vo.

2.9.1 Glucosinolate Analysis

The glucosinolate content and profile of the 80 lines of the 98-B-4 population, the parental lines and several commercial *Sinapis alba* and *Brassica napus* lines was determined as described by Raney and McGregor (1990) using gas chromatography. A 25 m by 0.32 mm column with a 0.52 µm Ultra1 (crosslinked Methyl Siloxane) coating was used. Additionally whole seed analysis of total glucosinolate content was determined by near infrared measurements (FOSS NIR) calibrated against the Federation of Oils, Seeds and Fats Association (FOSFA) extractions reference method and are expressed on a 8.5% moisture basis.

2.9.2 Whole Seed Analysis

Whole seed analysis for moisture, oil, protein, defatted protein and dry matter content of the 80 lines of the 98-B-4 population, the parental lines and several commercial *Sinapis alba* and *Brassica napus* lines, was determined by near infrared measurements (FOSS NIR Model 5000), calibrated against the FOSFA extractions reference method.

2.9.3 Fatty Acid Composition

Fatty acid composition of the 80 lines of the 98-B-4 population, the parental lines and several commercial *Sinapis alba* and *Brassica napus* lines was determined by the International Organization for Standardization method reference number ISO 5508:1990(E), Animal and vegetable fats and oils – Analysis by gas chromatography of methyl esters of fatty acids. A 15 m by 0.25 mm column with a 0.25 µm HP-Innowax (crosslinked polyethylene glycol) coating was used.

2.9.4 Half – Seed Fatty Acid Composition

Half-seed fatty acid analysis was conducted essentially as described by Downey and Harvey (1963) and Uppström and Johanson (1978). Seeds were placed on 9 cm Whatman #1 filter paper in 100 mm plastic petri dishes with sufficient water to saturate the paper. The seeds were then allowed to germinate at 25^0C for approximately 22 hours. One cotyledon was harvested at the time cracking of the seed coat was evident. Each harvested cotyledon was placed in a 12 x 75 culture tube (Corning) and allowed to dry for > 1 hour at 65^0C. Once dry 1 ml of methylating solution (20 gms of Sodium Methoxide in 1L of methanol) was added to the tubes. The cotyledon was crushed using a glass rod and 400 ul of hexane was added. The tube was capped, vortexed and left to stand in the dark for ½ hour. One ml of 1M NaCl was then added and the top layer of supernatant was removed and placed in a gas chromatography vial and capped immediately. Fatty acid composition was determined by the International Organization for Standardization method reference number ISO 5508:1990(E), Animal and vegetable fats and oils –

Analysis by gas chromatography of methyl esters of fatty acids. A 15-m by 0.25-mm column with a 0.25 μm HP-Innowax (crosslinked polyethylene glycol) coating was used.

2.10 Statistical Analysis

Statistical analysis was conducted using SAS version 6.12 (SAS Institute Inc., 1985). The mean data for each quality parameter was calculated for each line and Duncan's new multiple range test ($P<0.05$) was used to separate the means. A different letter grouping indicates that the mean results are statistically different from one another. Analysis of variance was performed for each quality component.

III. Results and Discussion

3.1 Flea Beetle Damage Ratings

The two DH populations (98-A-4 and 98-B-4) and their parental lines were screened for flea beetle antixenosis in the summer of 1999. Additionally, several *S. alba* and commercial *B. napus* varieties were included in these testing studies. Although both DH populations showed segregation for flea beetle resistance, only the 98-B-4 population was selected for further study, due to time and resource limitations. The 98-B-4 population was submitted for two rounds of flea beetle testing, as evidenced by the data "1999 (a) Scoring " and "1999 (b) Scoring " (Table 1). The 1999 (a) scoring was conducted in the spring of 1999 and the 1999 (b) scoring was conducted in late summer. Each testing round consisted of the 10 x 10 latin square design as described in Section 2.2. The data were analyzed as described in Section 2.3.

The higher level of resistance to flea beetle feeding in *S. alba* lines relative to the majority of the 98-B-4 *B. napus* population and parental lines is consistent with previous findings (Putnam, 1977; Lamb, 1984; Palaniswamy and Lamb, 1992; Palaniswamy *et al.*, 1997). Several lines of the 98-B-4 population did show damage scores similar to the scores recorded for the *S. alba* lines (Table 1). *Sinapis alba* damage scores ranged from 26.41 to 30.87 in the 1999 (a) round of testing. *Brassica napus* lines 98-B-4-247, 242, 243, 189, 184, 178, 176, 172, 107, 246 and 195 had 1999 (a)scores ranging from 24.87 to 39.95 and were ranked as resistant. The 1999 (b) damage scores for these particular lines were not as consistent as recorded for the *S. alba* cultivars. However, lines 98-B-4-189 and 176 reported similar damage scores in both rounds of testing (28.80/34.05 and 31.34/36/34 respectively). The 1999 (a) beetle scoring was positively correlated with the

Table 1: Mean flea beetle damage scorings of the 98-B-4 DH mapping population from tests conducted during the summer of 1999. Duncan's Multiple Range Test ($P<0.05$) was used to separate the means. Lines were then categorized as to a resistance level rating. R = Resistant, N = Neutral (not significantly different from Quantum), S = Susceptible.

Individual	1999 (a) Scoring	1999(a) Rating	1999(b) Scoring	1999(b) Rating
LK-001	49.09	N	46.40	N
94-99	50.95	N	50.95	N
98-B-4-242	24.87	R	44.33	N
98-B-4-243	26.50	R	58.34	N
98-B-4-189	28.80	R	34.05	R
98-B-4-176	31.34	R	36.34	R
98-B-4-107	33.01	R	53.08	N
98-B-4-246	33.84	R	66.26	N
98-B-4-195	35.86	R	48.05	N
98-B-4-187	36.39	N	40.00	N
98-B-4-178	36.58	R	47.18	N
98-B-4-190	36.91	N	59.68	N
98-B-4-247	37.10	R	n/a	
98-B-4-181	39.18	N	51.79	N
98-B-4-172	39.95	R	47.83	N
98-B-4-064	40.41	N	55.92	N
98-B-4-194	40.58	N	44.90	N
98-B-4-180	41.13	N	43.39	N
98-B-4-174	41.55	N	44.69	N
98-B-4-241	42.20	N	72.80	S
98-B-4-116	42.58	N	70.75	S
98-B-4-157	43.38	N	59.42	N
98-B-4-245	43.62	N	56.19	N
98-B-4-179	43.72	N	46.15	N
98-B-4-133	44.29	N	46.62	N
98-B-4-182	44.37	N	51.79	N
98-B-4-003	44.48	N	32.46	R
98-B-4-017	45.21	N	53.07	N
98-B-4-184	45.29	R	55.13	N
98-B-4-192	45.29	N	37.38	N
98-B-4-004	46.13	N	39.47	N
98-B-4-014	46.13	N	38.89	N
98-B-4-244	46.48	N	72.41	S
98-B-4-012	47.79	N	37.43	N
98-B-4-099	48.01	N	71.72	S
98-B-4-177	48.05	N	49.23	N
98-B-4-070	48.17	N	39.18	N
98-B-4-009	48.62	N	38.30	N
98-B-4-173	48.86	N	43.24	N
98-B-4-191	49.48	N	28.64	R
98-B-4-185	49.52	N	42.62	N
98-B-4-063	50.00	N	49.56	N
98-B-4-073	50.00	N	64.29	S
98-B-4-008	50.28	N	42.40	N
98-B-4-061	50.34	N	59.65	N
98-B-4-197	50.48	N	41.80	N
98-B-4-128	50.94	N	48.37	N
98-B-4-118	51.18	N	52.33	N
98-B-4-069	51.83	N	49.90	N
98-B-4-126	52.83	N	62.09	N
98-B-4-175	52.97	N	45.41	N
98-B-4-089	53.14	N	55.24	N
98-B-4-010	53.59	N	25.44	R
98-B-4-132	53.77	N	62.33	N
98-B-4-120	54.48	N	55.81	N
98-B-4-088	54.97	N	60.48	N
98-B-4-117	55.90	N	78.37	S

Individual	**1999 (a)** **Scoring**	**1999(a)** **Rating**	**1999(b)** **Scoring**	**1999(b)** **Rating**
98-B-4-109	57.58	N	66.12	N
98-B-4-047	57.88	N	54.61	N
98-B-4-112	58.33	N	30.87	R
98-B-4-059	58.90	N	46.71	N
98-B-4-108	59.34	N	55.46	N
98-B-4-127	60.85	N	75.12	S
98-B-4-111	61.11	N	40.98	N
98-B-4-110	61.36	N	36.34	N
98-B-4-092	61.93	N	86.66	S
98-B-4-113	63.13	S	44.26	N
98-B-4-067	63.61	S	62.34	S
98-B-4-068	64.14	S	48.70	N
98-B-4-101	64.20	N	77.02	S
98-B-4-135	66.21	S	67.39	S
98-B-4-103	68.18	S	93.18	S
98-B-4-081	68.32	S	40.00	N
98-B-4-090	70.16	S	49.76	N
98-B-4-084	70.42	S	65.66	S
98-B-4-072	71.20	S	45.02	N
98-B-4-065	71.47	S	51.08	N
98-B-4-102	71.59	S	95.20	S
98-B-4-080	71.73	S	53.10	N
98-B-4-022	74.32	S	44.96	N
98-B-4-074	76.18	S	58.33	N
98-B-4-104	78.98	S	80.81	S
BNA-013 *B. napus* cultivar	79.67	S	79.67	S
BNA-032 *B. napus* cultivar	33.78	R	40.95	N
BNA-083 *B. napus* cultivar	31.54	R	46.86	N
LKO-569	37.88	R	57.68	N
SAL-011 *S. alb* a cultivar	26.41	R	26.41	R
SAL-016 *S.alba* cultivar	28.04	R	26.63	R
SAL-027 *S. alba* cultivar	30.87	R	30.87	R
SAL-044 *S.alba* cultivar	27.04	R	21.76	R
Correlation 1999(a): 1999(b) = 0.43				

1999 (b) scoring (r=0.43) although it was a low correlation. Differences between the 1999 (a) and 1999 (b) scorings were presumably caused by two factors. First, there is substantial inherent variation in flea beetle ratings due to variation in the feeding rate of field-collected beetles (Lamb *et al*., 1993). Second, the feeding patterns of flea beetles collected in spring differ from feeding patterns of beetles collected in late summer (Lamb, 1988). Spring flea beetles attack young canola seedlings (in the cotyledon stage prior to true leaf development) whereas later season flea beetles seek the adult canola plants, specifically true leaves and pods (Palaniswamy and Lamb, 1992). Therefore the flea beetles captured for the second round of testing may have selected plants based on a different criteria than beetles collected for the first round of testing.

In previous years of testing (Appendix 1) LK-001 was rated as resistant and 94-99 was rated as susceptible or not significantly different from control. Damage ratings for the parental lines, LK-001 and 94-99 were not significantly different from each other or the check Quantum in the 1999 testing (Duncan's multiple range test, P<0.05). The level of flea beetle feeding was intense during the 1999 (a) testing and large differences among cultivars were difficult to detect. This has been found to be a problem with laboratory testing for flea beetle damage (Lamb, 1988).

Several lines within the 98-B-4 population had high scores of flea beetle damage (exceeding scores of both parental lines) and this damage was consistent over the two rounds of testing. Lines 98-B-4-135, 084, 102 and 104 had scores ranging from 66.21 to 78.98 in the early testing and 67.39 to 95.20 in the late testing and were considered to be susceptible (Table 1). Several other lines were not significantly different from their parental lines or the Quantum damage scores and were rated as neutral (Duncan's multiple range test, $P<0.05$). Based on this data the population was determined to segregate for flea beetle resistance.

3.2 RFLP Genetic Mapping

3.2.1 Parental Screening

Informative polymorphisms were detected between the resistant parental lines LK-001, LK-569, and the susceptible parental 94-99. Based on the degree of polymorphism, a further examination of the 98-A-4 and 98-B-4 mapping populations was warranted. Due to time and resource restraints, it was decided to concentrate on the 98-B-4 population. This study was based on the assumption that the flea beetle resistance is inherited from the *S. alba* background. Therefore it was anticipated that the resistance in the 98-B-4 population would segregate with a *Sinapis alba* allele. Of particular interest were the differences in banding patterns of the *S. alba* lines and the *B. napus* lines (Figure 3) as it could provide molecular marker information for flea beetle resistance.

3.2.2 Preliminary RFLP Probe Screening

A total of 57 cDNA probes were used to screen the parental lines of the 98-B-4 population and seven randomly selected lines from the population. A high degree of polymorphism was evident within the population. Six different enzyme digests were initially used but EcoRI provided the highest degree of polymorphism. The decision was made to continue screening using only EcoRI. Of the 57 probes used for all digests, 50 gave informative polymorphic loci between the LK-001 (resistant) and 94-99 (susceptible) individuals and within the 98-B-4 population.

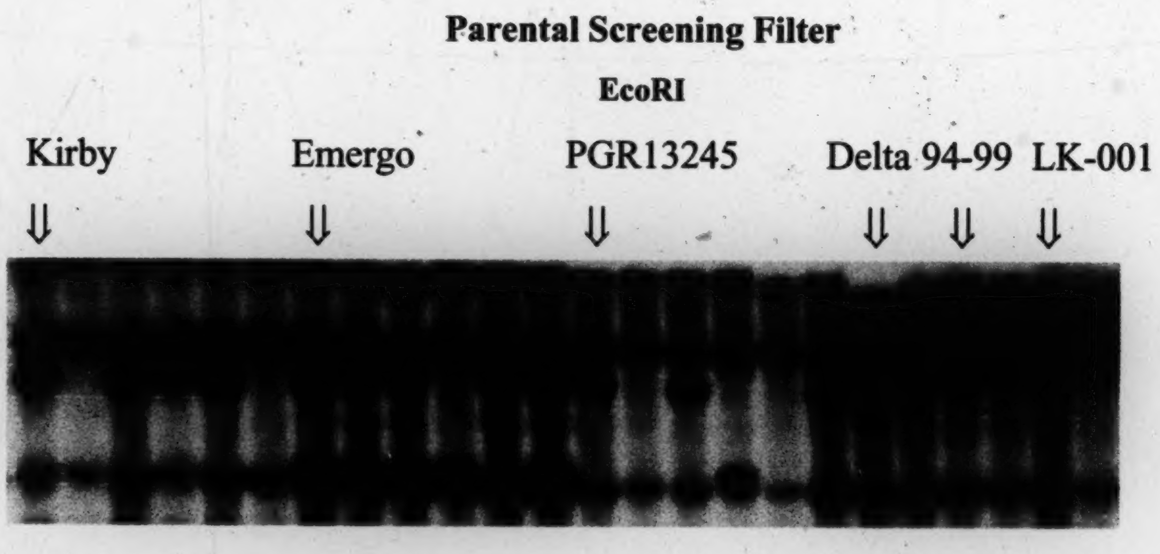

Parental Screening Filter
EcoRI
Kirby
Emergo
PGR13245
Delta
94-99
LK-001

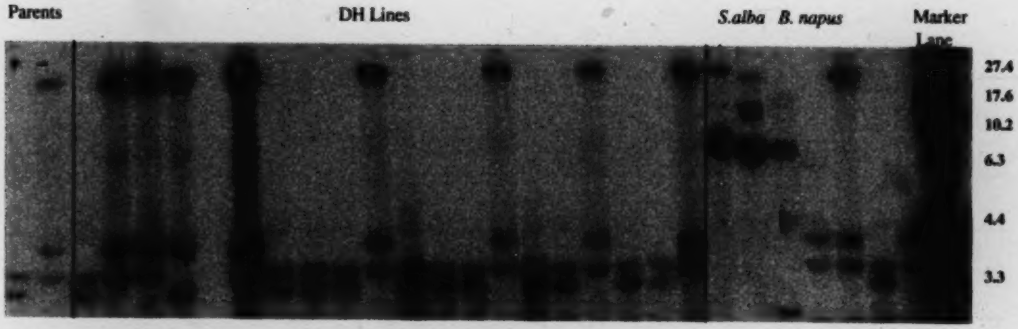
Figure 3: Parental screening filter showing differences in banding patterns of the *Sinapis alba* lines and the 98-B-4 parental lines. Six individuals were probed for each *S. alba* parent, whereas two individuals were tested for Delta, 94-99 and LK-001.

3.2.3 Genetic Mapping of 98-B-4 Population

3.2.3.1 Linkage Mapping

Seventy-eight cDNA clones or markers were used to probe the 98-B-4 population. Sixty-one of the 78 probes provided a total of 92 polymorphic loci and the results were used to generate a linkage map of the population. The first step in the generation of a genetic map from the RFLP markers is to score polymorphisms on the autoradiograph. These banding patterns result from the hybridization of the labeled probe to the digested genomic DNA of the two parents and each individual member of the mapping population. In the case of a doubled haploid population and recombinant inbred lines, polymorphic alleles at a locus between parents will generally segregate among the progeny in a disomic pattern with each individual inheriting one parental allele or the other. As data from more and more probes are collected, it will be observed that certain pairs of markers are inherited in a similar fashion among members of the mapping population (Figure 4, Figure 5). This indicates linkage of the markers. The probability of markers being linked is given by the LOD, which is the ratio of the probability that a marker is linked to the probability that it is not linked. A marker with a LOD score of 3.0 (10^3 times more likely to be linked to its neighbors than unlinked) is generally considered to be strong evidence for linkage (Lander *et al.*, 1987).

Linkage analysis established 18 linkage groups with 66 polymorphic loci (Figure 6), combining 3 or more loci using the linkage software MAPMAKER/EXP 3.0b (Lander *et al.*, 1987) and manual analyses. Twenty-six loci remained unlinked. This methodology is comparable to that used to construct the RFLP linkage maps for many species

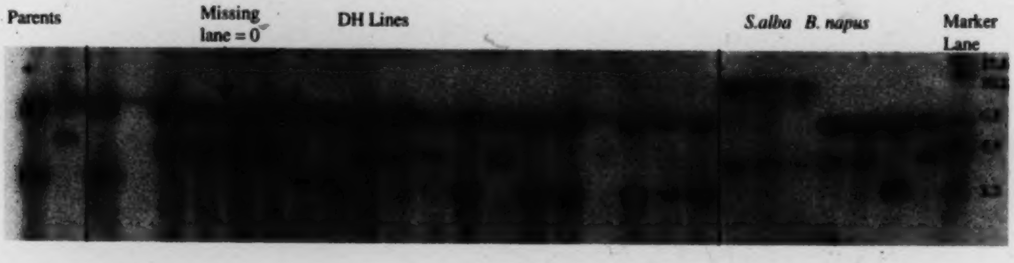


Autoradiograph of ptglh12a

Parents
DH Lines
S.alba
B. napus
Marker Lane
27.4
17.6
10.2
6.3
4.4
3.3

Scoring Data:

+ - - ? 0 - + + + - + + + - + + - + + -



Autoradiograph of pwg3h8

Parents
Missing lane = 0
DH Lines
S.alba
B. napus
Marker Lane

Scoring Data:

+ 0 - - - - + + - - - + - + + 0 + + +

Figure 4: Autoradiographs for cDNA/genomic probes ptg1h12a and pwg3h8 on the 98-B-4 *B. napus* DH mapping population. + allele inherited from the LK-001 parent and – allele inherited from the 94-99 parent. The RFLP patterns are typical of disomic inheritance. Each individual of the 98-B-4 DH mapping population received a single parental allele at each polymorphic locus, indicating normal homologous chromosome pairing at meiosis. ? Individual is carrying allele from LK-001 **and** 94-99 parent due to a translocation event. Numbers to the right are size standards in kilobase pairs(kb).

A: Integrated Scoring Data

```
ptg1h12a   + - - ? 0 - + + + - + + + - + + - + + -
pec2c7     + - - - 0 + - + + - - - + - + + - + + +
pwg3h8     + 0 - - 0 - - + + - - - + - + + 0 + + +
pwg4h5     + - - - 0 - - + + - - - + - - - - - + +
```

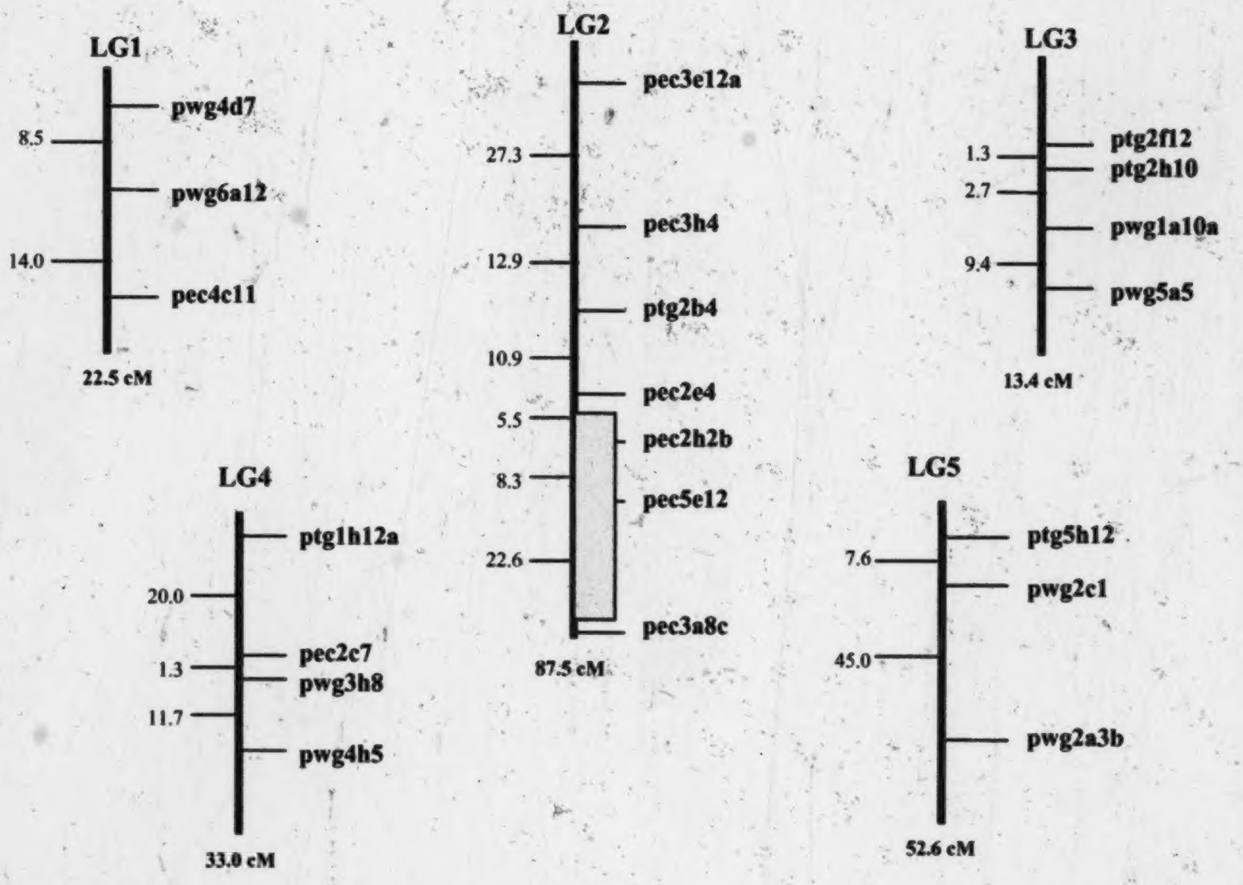
B: Mapmaker Output

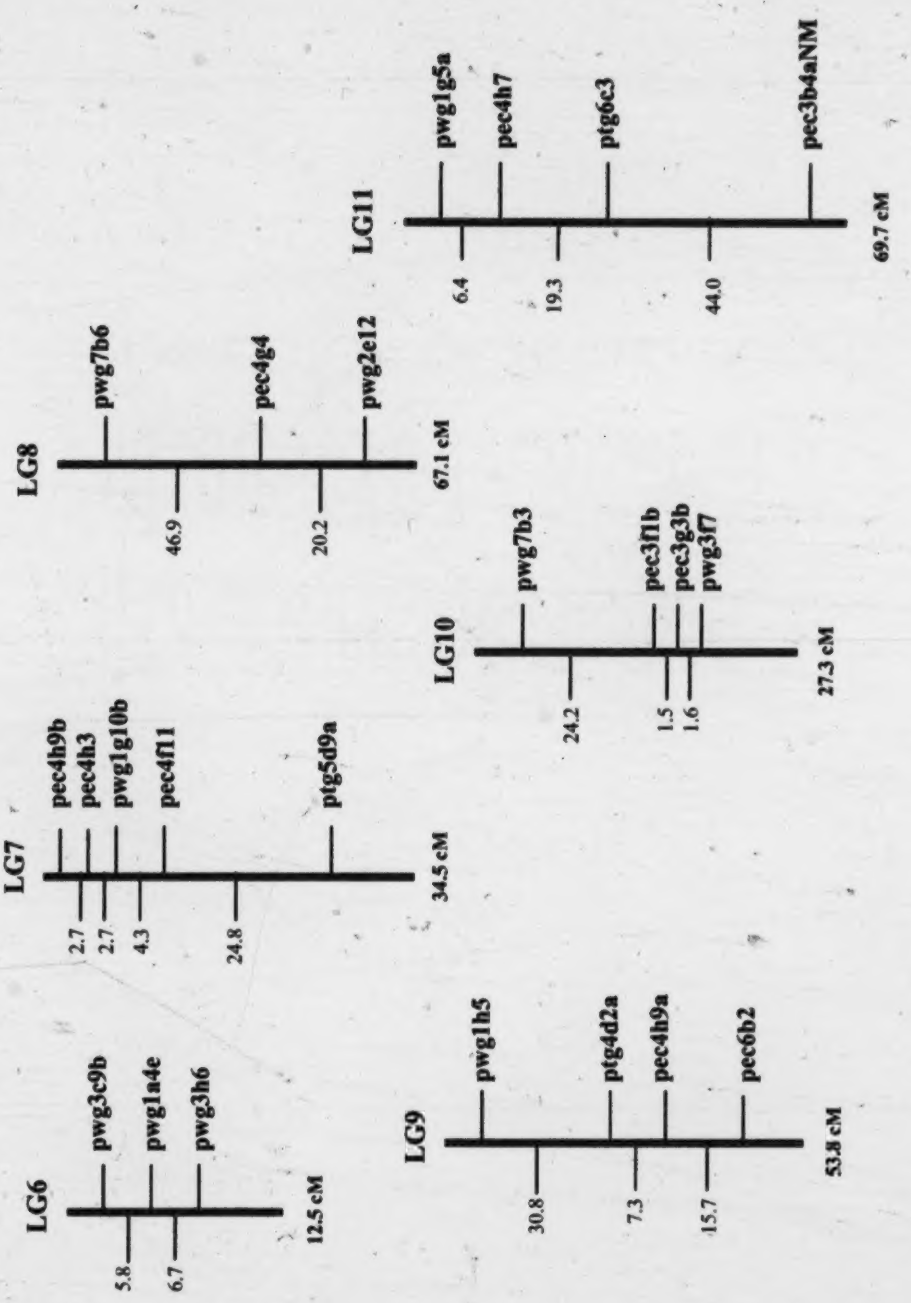
```
Map Linkage group 4:

              Markers                 Distance

              15 ptg1h12a             17.1 cM

               4 pec2c7                1.3 cM

              30 pwg3h8               10.6 CM

               5 pwg4h5               -------

                                      28.9 cM 4 markers

                                      log-likelihood = -105.19
```

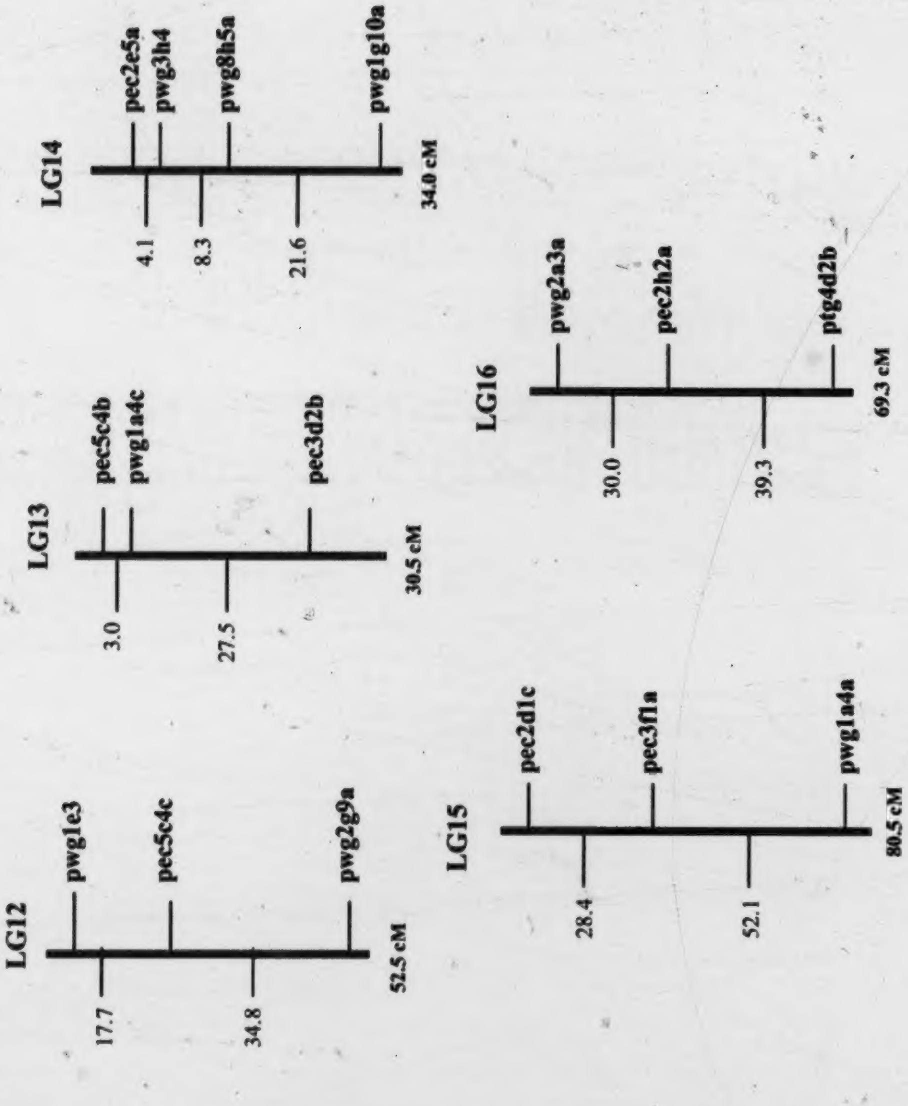
Figure 5: A: Inheritance pattern of the alleles at the ptg1h12a and pwg3h8 loci is consistent with that of the flanking markers.

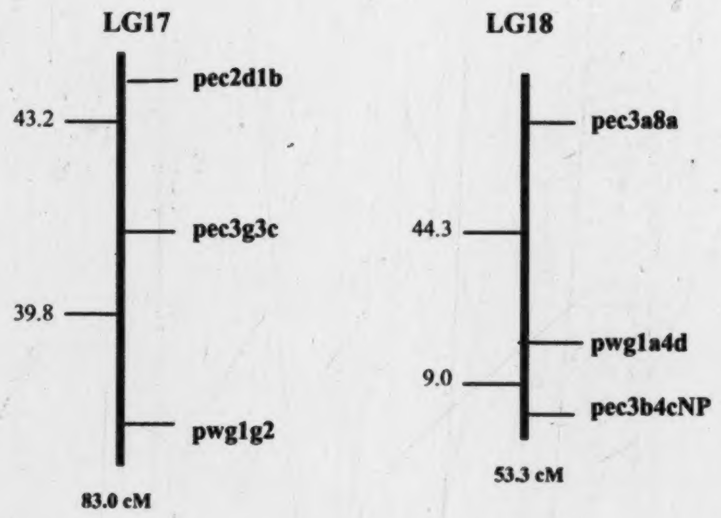
B: Map of this linkage group generated with MAPMAKER. This particular order of markers is $10^{105.19}$ times more likely than any other order.

Figure 6: A genetic linkage map of the 98-B-4 *B. napus* DH population based upon segregation of 66 loci as assigned by MAPMAKER/EXP version 3.0b (Lander *et al.*, 1987) and through additional manual manipulations. Vertical lines represent the linkage groups with loci represented by the code for the appropriate probe to the right. All loci separated by recombination are shown as distinct horizontal bars on linkage groups with distances expressed in centimorgans (cM) to the left. The dark boxed region on Linkage group 2, between markers pec2e4 and pec3a8c, indicates the region of a putative QTL for flea beetle resistance as assigned by MAPMAKER/QTL 1.1b (Lander *et al.*, 1987).


LG1
pwg4d7
8.5
pwg6a12
14.0
pec4c11
22.5 cM
LG2
pec3e12a
27.3
pec3h4
12.9
ptg2b4
10.9
pec2e4
5.5
pec2h2b
8.3
pec5e12
22.6
pec3a8c
87.5 cM
LG3
ptg2f12
1.3
ptg2h10
2.7
pwg1a10a
9.4
pwg5a5
13.4 cM
LG4
ptg1h12a
20.0
pec2c7
1.3
pwg3h8
11.7
pwg4h5
33.0 cM
LG5
ptg5h12
7.6
pwg2c1
45.0
pwg2a3b
52.6 cM


LG6
pwg3c9b
pwg1a4e
pwg3h6
5.8
6.7
12.5 cM
LG7
pec4h9b
pec4h3
pwg1g10b
pec4f11
ptg5d9a
2.7
2.7
4.3
24.8
34.5 cM
LG8
pwg7b6
pec4g4
pwg2e12
46.9
20.2
67.1 cM
LG9
pwg1h5
ptg4d2a
pec4h9a
pec6b2
30.8
7.3
15.7
53.8 cM
LG10
pwg7b3
pec3f1b
pec3g3b
pwg3f7
24.2
1.5
1.6
27.3 cM
LG11
pwg1g5a
pec4h7
ptg6c3
pec3b4aNM
6.4
19.3
44.0
69.7 cM


LG12
pwg1e3
pec5c4c
pwg2g9a
17.7
34.8
52.5 cM
LG13
pec5c4b
pwg1a4c
pec3d2b
3.0
27.5
30.5 cM
LG14
pec2e5a
pwg3h4
pwg8h5a
pwg1g10a
4.1
8.3
21.6
34.0 cM
LG15
pec2d1c
pec3f1a
pwg1a4a
28.4
52.1
80.5 cM
LG16
pwg2a3a
pec2h2a
ptg4d2b
30.0
39.3
69.3 cM


LG17
pec2d1b
43.2
pec3g3c
39.8
pwg1g2
83.0 cM
LG18
pec3a8a
44.3
pwg1a4d
9.0
pec3b4cNP
53.3 cM

Unlinked:

pwg1a10b
pec2d1a
pec3d2a
pec3b2
pec3e12b
pwg1a4b
pwg8h5b
pec2d8
ptg5d9b
pwg2g9b
pec3a8b
pec5c4d
pwg9e9
pec2c12
pwg6h1
pwg3c9a

pwg2a3c
pwg8h5c
pec3b4b
pwg3g11
pec2e5b
pec3b12
pwg7d9
pec3g3a
pec5c4aNP

including *B. juncea* (Cheung *et al.*, 1997) and *B. napus* (Sharpe *et al.*, 1995; Parkin *et al.*, 1995).

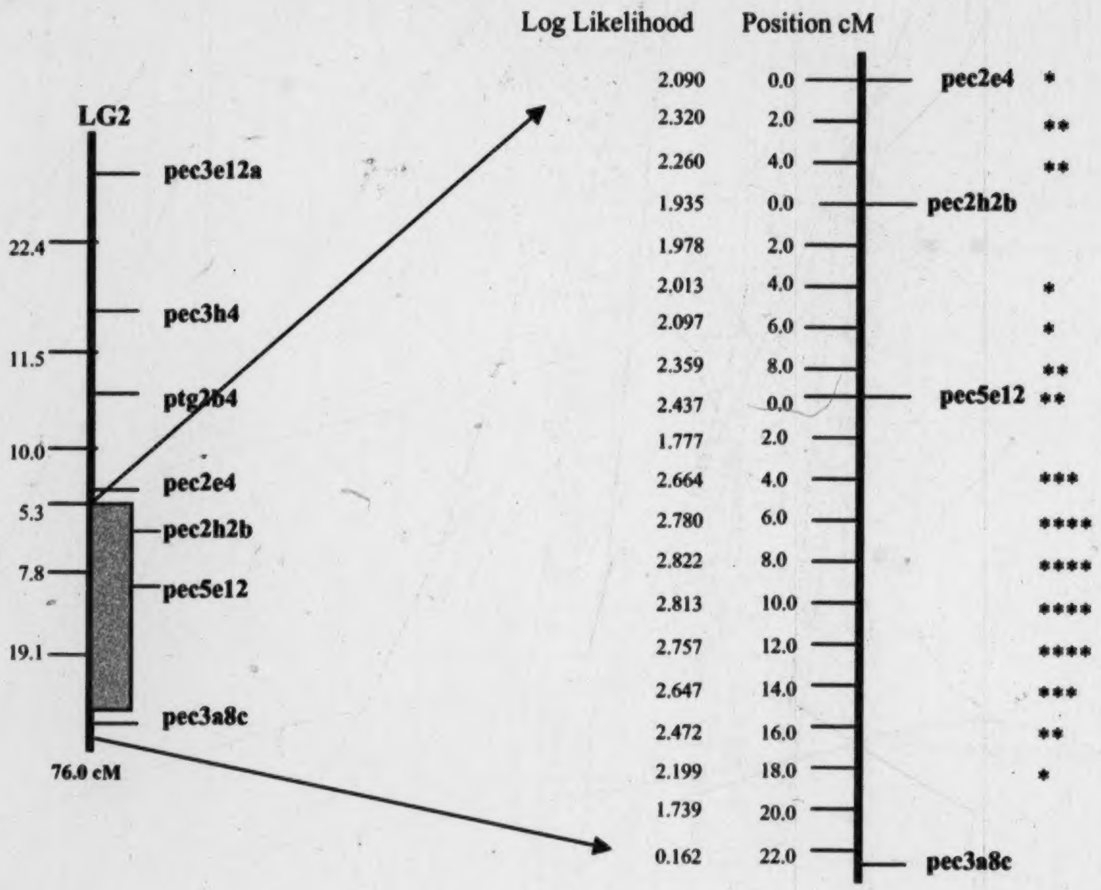
3.2.3.2 QTL Analysis

Once linkage groups were established, the flea beetle rating data 1999 (a) was entered into MAPMAKER/QTL 1.1b (Lander *et al.*, 1987) for analysis. MAPMAKER/QTL assumes that the values of the quantitative trait (in this case flea beetle rating 1999 (a)) vary across the population following a normal distribution. MAPMAKER/QTL then computes QTL likelihood plots covering the entire genome. These graphs visually present the regions of the genome which are likely to contain putative QTLS and the strength of the data supporting the idea that particular QTLs exist and their likely position (Figure 7). The initial QTL analysis detected a putative QTL for flea beetle resistance on a region of linkage group 2, between marker pec2e4 and pec3a8c. This is the typical result of a data set which does not contain sufficient information to resolve QTL position more accurately (Lander *et al.*, 1987).

3.2.3.3 Allelic Distortion

Appendix 2 provides a summary of allelic frequency within the 98-B-4 DH mapping population. Differences between the observed and expected frequencies could be due to chance alone or other factors may be involved (Lander *et al.*, 1987). The Chi-square analysis (X^2) tests the significance of these allelic frequencies. Linkages 14 and 15 are significantly (X^2, $P<0.05$) distorted in favor of the LK-001 (resistant) parental allele, which leads us to conclude that the observed values are unlikely to arise from

Figure 7: An expanded view of the region of linkage group 2 containing a putative QTL for flea beetle rating 1999 (a). Position cM = position as MAPMAKER/QTL analysis steps along the genome at 2 cM intervals between each marker. Log likelihood = strength of data supporting the idea that this region of the genome contains a putative QTL affecting flea beetle resistance 1999 (a), indicated by log likelihoods greatly exceeding our threshold of 2.0. The output for these regions is indicated by "*" characters printed next to each data line. "*" represents a log likelihood >2.0; "**" represents a log likelihood > 2.25; "***" represents a log likelihood >2.50; "****" represents a log likelihood >2.75.


LG2
pec3e12a
22.4
pec3h4
11.5
ptg2b4
10.0
pec2e4
5.3
pec2h2b
7.8
pec5e12
19.1
pec3a8c
76.0 cM
Log Likelihood
Position cM
2.090 0.0 pec2e4 *
2.320 2.0 **
2.260 4.0 **
1.935 0.0 pec2h2b
1.978 2.0
2.013 4.0 *
2.097 6.0 *
2.359 8.0 **
2.437 0.0 pec5e12 **
1.777 2.0
2.664 4.0 ***
2.780 6.0 ****
2.822 8.0 ****
2.813 10.0 ****
2.757 12.0 ****
2.647 14.0 ***
2.472 16.0 **
2.199 18.0 *
1.739 20.0
0.162 22.0 pec3a8c

chance. Linkages 16 and 18 are also significantly (X^2, $P<0.05$) distorted but in favor of the parental allele 94-99 (susceptible).

Allelic distortion is a common observation in populations developed via microspore culture and has been reported in maize (Bentolila *et al.*, 1992), barley (Graner *et al.*, 1991; Thompson *et al.*, 1991), *Brassica oleracea* (Bohuon, 1995), and *Brassica napus* (Ferreira *et al.*, 1994; Mayerhofer *et al.*, 1997; Parkin and Lydiate, 1997). Bohuon (1995) and Parkin and Lydiate (1997) demonstrated that segregation distortion in the microspore-derived populations was the result of inadvertent viability selection during the tissue culture process, and had no significant effect on genetic analysis. The data presented here indicates selection for different alleles that is not specific for one parent or the other. Segregation distortion as reported is often observed in crosses between parents where the two parents are quite genetically distinct. This is particularly true when double haploid mapping populations are used, strongly indicating that selection is occurring during the tissue culture process for specific alleles or chromosomes.

QTL alleles for both pest resistance and susceptibility have been identified in other species. Significant differences in allelic frequencies have been found in natural populations of the cereal cyst nematode when tested on resistant and susceptible cultivars of cereals (Lasserre *et al.*, 1996). Ibrahim *et al.* (1996) found directional changes of allelic frequencies at two loci that were involved in mildew resistance in barley. In some cases, QTL alleles that increase pest susceptibility have been found to be dominant over alleles that increase resistance while in other cases, the opposite is true (Gebhardt, 1999). In this study the QTL for flea beetle resistance mapped to a region of the chromosome that also displayed segregation distortion. On linkage group two, the 94-99 (susceptible) allele was more favored at the pec3a8c marker, however, there is no reason to believe that there is a relationship between the allele and flea beetle susceptibility.

3.3 Quality Analysis

The 98-B-4 population and parents were evaluated for seed quality characteristics. A subset of the population was also submitted for half seed cotyledon fatty acid analysis in order to determine individual plant fatty acid levels. Data analysis was performed in SAS 6.12 (SAS Institute, 1996). SAS data analyses were used to determine if the parental lines LK-001 (resistant) and 94-99 (susceptible) were significantly different from each other for each of the quality parameters. If the parental lines were found to be significantly different, then a further evaluation of differences between individual lines and parental lines was carried out. These significantly different lines were then compared based on their flea beetle ratings to determine if there was a relationship between the particular quality trait and the flea beetle rating.

3.3.1 Glucosinolate Content

Total glucosinolates and glucosinolate profiles were determined for each line of the 98-B-4 population, parental lines and several *S. alba* and *B. napus* cultivars. Duplicate whole seed samples were run for each individual and the two readings were averaged. A subset of this data that highlights a cross section of the population is presented in Table 2 while the entire data set is provided in Appendix 3. Analysis of variance (Appendix 4) and Duncan's Multiple Range Test ($P<0.05$) were run on the raw glucosinolate profile data. Significant differences ($P<0.05$) between the parental lines of

Table 2: Summary of whole seed glucosinolate profile of the 98-B-4 DH mapping population. Duncan's Multiple Range Test (P<0.05) was used to separate the means. A different letter group indicates that the mean results are statistically different from one another.

Individual	1999(a) Scoring	1999(a) Rating	Total Alkenyl (Aliphatics) um/g	Duncan's Multiple Range Alkenyl	Total Indolyl um/g	Duncan's Multiple Range Indolyl	Total Glucosinolate um/g oil free meal	Duncan's Multiple Range Glucosinolate	Total Allyl um/g	Duncan's Multiple Range Allyl	3-Butenyl um/g	Duncan's Multiple Range 3-Butenyl	4-Pentenyl um/g	Duncan's Multiple Range 4-Pentenyl
LK-001	49.09	N	8.70	A	13.51	A	22.21	A	0.10	A	2.55	A	0.69	A
94-99	50.95	N	7.89	A	9.42	B	17.31	A	0.19	A	1.55	A	0.53	A
98-B-4-242	24.87	R	10.96	A	11.77	A	22.73	A	0.14	A	3.08	A	1.27	A
98-B-4-243	26.50	R	16.76	C	11.26	AB	28.02	A	0.12	A	4.43	A	3.14	B
98-B-4-189	28.80	R	6.91	A	11.23	AB	18.15	A	0.05	A	2.19	A	0.83	A
98-B-4-176	31.34	R	5.56	A	13.17	A	18.73	A	0.00	A	1.91	A	0.72	A
98-B-4-107	33.01	R	18.38	C	9.98	B	28.36	B	0.12	A	3.21	A	2.22	A
98-B-4-246	33.84	R	15.24	C	6.65	B	21.89	A	0.14	A	2.82	A	2.33	B
98-B-4-195	35.86	R	7.48	A	7.66	B	15.14	A	0.08	A	2.49	A	1.16	A
98-B-4-187	36.39	N	5.25	A	6.57	B	11.83	A	0.11	A	1.14	A	0.43	A
98-B-4-178	36.58	R	10.12	A	7.18	B	17.30	A	0.04	A	2.34	A	1.26	A
98-B-4-190	36.91	N	11.59	A	11.08	A	22.67	A	0.13	A	2.07	A	0.67	A
98-B-4-247	37.10	R	10.61	A	7.90	B	18.51	A	0.15	A	2.75	A	1.29	A
98-B-4-181	39.18	N	5.14	D	5.40	A	10.54	B	0.00	A	1.38	A	0.72	A
98-B-4-172	39.95	R	6.56	A	13.82	A	20.38	A	0.11	A	1.44	A	0.38	A
98-B-4-111	61.11	N	8.55	A	10.98	AB	19.53	A	0.17	A	3.04	A	0.87	A
98-B-4-110	61.36	N	2.65	D	7.00	B	9.65	B	0.15	A	0.83	A	0.28	A
98-B-4-092	61.93	N	12.52	A	14.41	A	26.93	A	0.07	A	4.05	A	1.17	A
98-B-4-113	63.13	S	8.47	A	11.86	A	20.33	A	0.09	A	3.60	A	0.97	A
98-B-4-067	63.61	S	0.71	E	4.83	A	5.54	B	0.00	A	0.27	A	0.02	A
98-B-4-068	64.14	S	7.52	A	9.30	B	16.82	A	0.04	A	1.86	A	1.03	A
98-B-4-101	64.20	N	3.77	D	11.64	A	15.41	A	0.08	A	1.40	A	0.47	A
98-B-4-135	66.21	S	3.49	D	11.36	A	14.85	A	0.00	A	1.30	A	0.20	A
98-B-4-103	68.18	S	6.79	A	11.03	A	17.82	A	0.12	A	2.08	A	1.21	A
98-B-4-081	68.32	S	3.15	D	10.57	B	13.72	A	0.00	A	0.94	A	0.17	A
98-B-4-022	74.32	S	4.77	D	11.39	A	16.15	A	0.00	A	1.95	A	0.67	A
98-B-4-074	76.18	S	7.08	A	12.14	A	19.21	A	0.00	A	2.08	A	0.96	A
98-B-4-104	78.98	S	5.97	D	12.73	A	18.70	A	0.09	A	1.82	A	0.84	A
SAL-011 (*S.alba*)	26.41	R	2.20	D	1.11	A	3.31	B	0.03	A	0.06	A	0.00	A
SAL-016 (*S.alba*)	28.04	R	3.59	D	1.52	A	5.11	B	0.07	A	0.09	A	0.00	A
SAL-027 (*S.alba*)	30.87	R	2.64	D	1.39	A	4.03	B	0.13	A	0.16	A	0.00	A
SAL-044 (*S.alba*)	27.04	R	2.71	D	1.49	A	4.21	B	0.12	A	0.18	A	0.00	A
Check (Average)			13.97	C	12.27	A	26.24	A	1.30	B	4.19	A	0.89	A

Individual	1999(a) Scoring	1999(a) Rating	2-Hydroxy 3-Butenyl	Duncan's Multiple Range	2-Hydroxy 4-Pentenyl	Duncan's Multiple Range	3-Indolylmethyl	Duncan's Multiple Range	4-Hydroxy 3-indolylmethyl	Duncan's Multiple Range
			um/g	Hydroxy/Butenyl	um/g	Hydroxy/Pentenyl	um/g	Indolylmethyl	um/g	Hyrdoxy/Indolylmethyl
LK-001	49.09	N	5.15	A	0.20	A	1.93	A	11.58	A
94-99	50.95	N	5.18	A	0.45	A	1.19	B	8.23	B
98-B-4-242	24.87	R	6.05	A	0.42	A	0.57	B	11.20	AB
98-B-4-243	26.50	R	8.24	A	0.83	A	1.11	B	10.15	AB
98-B-4-189	28.80	R	3.63	A	0.21	A	0.83	B	10.40	AB
98-B-4-176	31.34	R	2.78	A	0.13	A	1.92	A	11.25	AB
98-B-4-107	33.01	R	11.07	B	1.77	B	0.52	C	9.47	AB
98-B-4-246	33.84	R	8.39	B	1.57	B	0.28	C	6.37	B
98-B-4-195	35.86	R	3.38	A	0.37	A	0.51	C	7.15	B
98-B-4-187	36.39	N	3.37	A	0.20	A	0.20	C	6.38	B
98-B-4-178	36.58	R	6.02	A	0.46	A	0.29	C	6.89	B
98-B-4-190	36.91	N	8.07	A	0.65	A	2.24	A	8.84	B
98-B-4-247	37.10	R	5.76	A	0.67	A	0.34	C	7.56	B
98-B-4-181	39.18	N	2.88	A	0.16	A	0.26	C	5.14	B
98-B-4-172	39.95	R	4.37	A	0.26	A	0.48	C	13.34	A
98-B-4-111	61.11	N	4.19	A	0.28	A	0.71	B	10.27	AB
98-B-4-110	61.36	N	1.30	C	0.08	A	0.20	C	6.81	B
98-B-4-092	61.93	N	7.08	A	0.14	A	1.24	B	13.16	A
98-B-4-113	63.13	S	3.66	A	0.15	A	0.90	B	10.96	AB
98-B-4-067	63.61	S	0.42	C	0.00	A	0.05	C	4.77	B
98-B-4-068	64.14	S	4.15	A	0.44	A	0.33	C	8.98	B
98-B-4-101	64.20	N	1.74	C	0.08	A	1.65	A	9.99	AB
98-B-4-135	66.21	S	1.92	A	0.06	A	0.55	B	10.81	AB
98-B-4-103	68.18	S	3.00	A	0.39	A	1.24	B	9.79	AB
98-B-4-081	68.32	S	2.02	A	0.02	A	0.25	C	10.32	AB
98-B-4-022	74.32	S	2.10	A	0.05	A	0.52	C	10.87	AB
98-B-4-074	76.18	S	3.77	A	0.26	A	0.68	B	11.45	AB
98-B-4-104	78.98	S	2.94	A	0.27	A	1.44	B	11.29	AB
SAL-011 (*S.alba*)	26.41	R	2.02	A	0.08	A	0.34	C	0.78	C
SAL-016 (*S.alba*)	28.04	R	3.43	A	0.00	A	0.43	C	1.08	C
SAL-027 (*S.alba*)	30.87	R	2.30	A	0.05	A	0.38	C	1.01	C
SAL-044 (*S.alba*)	27.04	R	2.33	A	0.08	A	0.36	C	1.13	C
Check (Average)			7.23	A	0.37	A	1.21	B	11.05	AB

LK-001 (resistant) and 94-99 (susceptible) for total indolyl glucosinolates, 3-indolylmethyl glucosinolates and 4-hydroxy-3-indolylmethyl glucosinolates were observed. The R-square values (0.88 to 0.92) for the analyses of the individual glucosinolate components was high, however, the coefficient of variance (C.V.) was high (13.26 to 29.16) (Appendix 4). Significant differences ($P<0.05$) between the data observations for 94-99 (susceptible) and resistant individuals may suggest a relationship between flea beetle resistance and glucosinolates.

Of those lines that were resistant to flea beetles, four lines 98-B-4-107, 176, 195 and 246 were also significantly different from the parental line 94-99 (susceptible) (Duncan's multiple range test, $P<0.05$) for 3-indolylmethyl glucosinolate. However, there is a poor correlation (0.04) between flea beetle resistance and 3-indolylmethyl glucosinolate. In addition, other resistant lines, 98-B-4-172, 189, 242 and 243 were not significantly different from 94-99 (Duncan's multiple range test, $P<0.05$).

The *S. alba* lines show a glucosinolate profile different from the *B. napus* parental lines of the 98-B-4 population. This is consistent with previous studies of the glucosinolate profile of *S. alba* (Horn and Vaughn, 1983). Lines 98-B-4-108 and 245 (Appendix 3) have total alkyls similar to *S. alba* cultivars and much lower than their parental lines. The remainder of their profiles appears to be intermediate between *S. alba* and the parental lines. Lines which were identified with similar resistance to flea beetle feeding as *S. alba* (98-B-4-242, 243, 189, 176, 107, 246 and 195) show a glucosinolate profile more typical of *B. napus*. This supports earlier findings that there is no consistent relationship between total seed glucosinolates and crop resistance to insect attack (Åhman, 1982; Rawlinson and Williams, 1991).

From the data provided in this thesis (Appendix 3), the average total aliphatic glucosinolate level for *S. alba* cultivars (Sal011, 016, 027, 044) is 2.79. Mapping population lines that were rated as resistant (98-B-4-107, 176, 189, 195, 242, 243, 246) have total aliphatic glucosinolates levels ranging from 5.56 to 18.38, and there is a negative correlation between the early flea beetle ratings and the total aliphatic glucosinolates (r=-0.02). This also supports the theory that total seed glucosinolates do not impact plant-insect interactions.

3.3.2 Oil and Protein Content

The 98-B-4 population was subsampled and analyzed for oil and protein whole seed basis using near infrared (NIR). Two samples were tested for each individual and the data were averaged to provide a mean result for each line. Table 3 presents a summary table of the means for the NIR determination for moisture, oil, protein, defatted protein, total glucosinolate and dry matter content of whole seeds of the 98-B-4 population as well as parental lines and several *S. alba* cultivars. The data set is presented in Appendix 5. Analysis of variance (Appendix 4) and Duncan's Multiple Range Test (P<0.05) were run on the raw NIR data. Significant differences between the data observations for 94-99 (susceptible) and resistant individuals may suggest a relationship between flea beetle resistance and whole seed components.

Parental lines LK-001 (resistant) and 94-99 (susceptible) were significantly different (P<0.05) for whole seed protein and 94-99 (susceptible) was also significantly

Table 3: Summary of whole seed NIR quality analysis of the 98-B-4 DH mapping population. Duncan's Multiple Range Test ($P<0.05$) was used to separate the means. A different letter group indicates that the mean results are statistically different from one another.

Individual	1999(a) Scoring	1999(a) Rating	% Moisture	% Oil	Duncan's Multiple Range Oil	% Protein	Duncan's Multiple Range Protein	% Defatted Protein	Duncan's Multiple Range Defatted Protein	Total Glucosinolate 8.5% moisture basis	% Dry Matter	Duncan's Multiple Range Dry Matter
LK-001	49.09	N	6.15	38.12	A	30.02	A	48.09	A	16.68	93.83	A
94-99	50.95	N	6.20	37.33	A	31.82	B	50.26	A	11.86	93.78	A
98-B-4-242	24.87	R	5.59	44.82	B	26.78	A	48.15	A	9.18	94.38	B
98-B-4-243	26.50	R	5.81	43.20	B	27.49	B	47.97	A	16.56	94.14	C
98-B-4-189	28.80	R	5.93	41.74	B	27.70	B	46.59	A	10.46	94.06	C
98-B-4-176	31.34	R	6.02	39.96	A	30.05	A	50.06	A	8.91	94.00	C
98-B-4-107	33.01	R	5.20	40.99	B	28.55	B	48.02	A	14.89	94.82	A
98-B-4-246	33.84	R	5.37	44.02	B	27.45	B	48.27	A	9.29	94.63	D
98-B-4-195	35.86	R	5.90	41.38	A	30.01	A	51.15	A	7.54	94.09	C
98-B-4-187	36.39	N	5.25	45.93	B	26.04	A	47.57	A	5.54	94.71	A
98-B-4-178	36.58	R	5.67	40.65	A	30.35	A	50.50	A	9.84	94.37	B
98-B-4-190	36.91	N	5.93	41.94	A	27.91	B	48.20	A	15.80	94.04	C
98-B-4-247	37.10	R	5.71	42.50	A	28.41	B	48.96	A	7.46	94.29	B
98-B-4-181	39.18	N	5.65	40.91	A	28.90	B	48.51	A	7.30	94.36	B
98-B-4-172	39.95	R	5.75	43.05	B	27.03	B	46.90	A	7.27	94.24	B
98-B-4-111	61.11	N	5.59	41.18	A	29.78	A	51.31	A	13.76	94.41	B
98-B-4-110	61.36	N	4.99	43.51	B	28.10	B	49.55	A	7.43	94.99	E
98-B-4-092	61.93	N	5.37	41.78	A	29.91	A	52.54	A	15.82	94.62	D
98-B-4-113	63.13	S	5.24	42.08	A	28.74	A	49.52	A	11.95	94.75	D
98-B-4-067	63.61	S	4.92	43.83	B	26.90	B	47.85	A	7.39	95.07	F
98-B-4-068	64.14	S	5.25	40.73	A	28.44	B	47.97	A	11.31	94.81	A
98-B-4-101	64.20	N	5.20	40.66	A	29.31	A	50.07	A	12.42	94.82	A
98-B-4-135	66.21	S	5.58	43.40	B	28.58	C	50.58	A	6.15	94.41	B
98-B-4-103	68.18	S	5.48	42.48	A	30.03	A	52.61	B	8.94	94.52	B
98-B-4-081	68.32	S	4.96	42.78	A	28.36	B	49.49	A	10.09	95.02	F
98-B-4-022	74.32	S	5.31	39.44	A	31.00	B	51.27	A	12.23	94.70	D
98-B-4-074	76.18	S	5.23	41.65	A	29.58	A	51.36	A	11.45	94.78	D
98-B-4-104	78.98	S	5.27	42.38	A	27.71	B	47.99	A	11.87	94.75	D
SAL-011 (*S.alba*)	26.41	R	7.09	25.53	C	37.63	D	52.01	A	39.86	92.81	D
SAL-016 (*S.alba*)	28.04	R	6.48	30.49	C	31.28	B	42.85	C	40.28	93.43	G
SAL-027 (*S.alba*)	30.87	R	6.50	29.86	C	33.39	E	47.34	A	38.55	93.44	G
SAL-044 (*S.alba*)	27.04	R	6.67	28.83	C	34.92	F	49.78	A	36.55	93.25	D
2000 Industry Results												
Canola				43.10		21.20				10.10		
Yellow Mustard				30.20		32.20				n/a		

Figure 8: Scatter Plot of 1999(a) Flea Beetle Rating vs % Oil Content

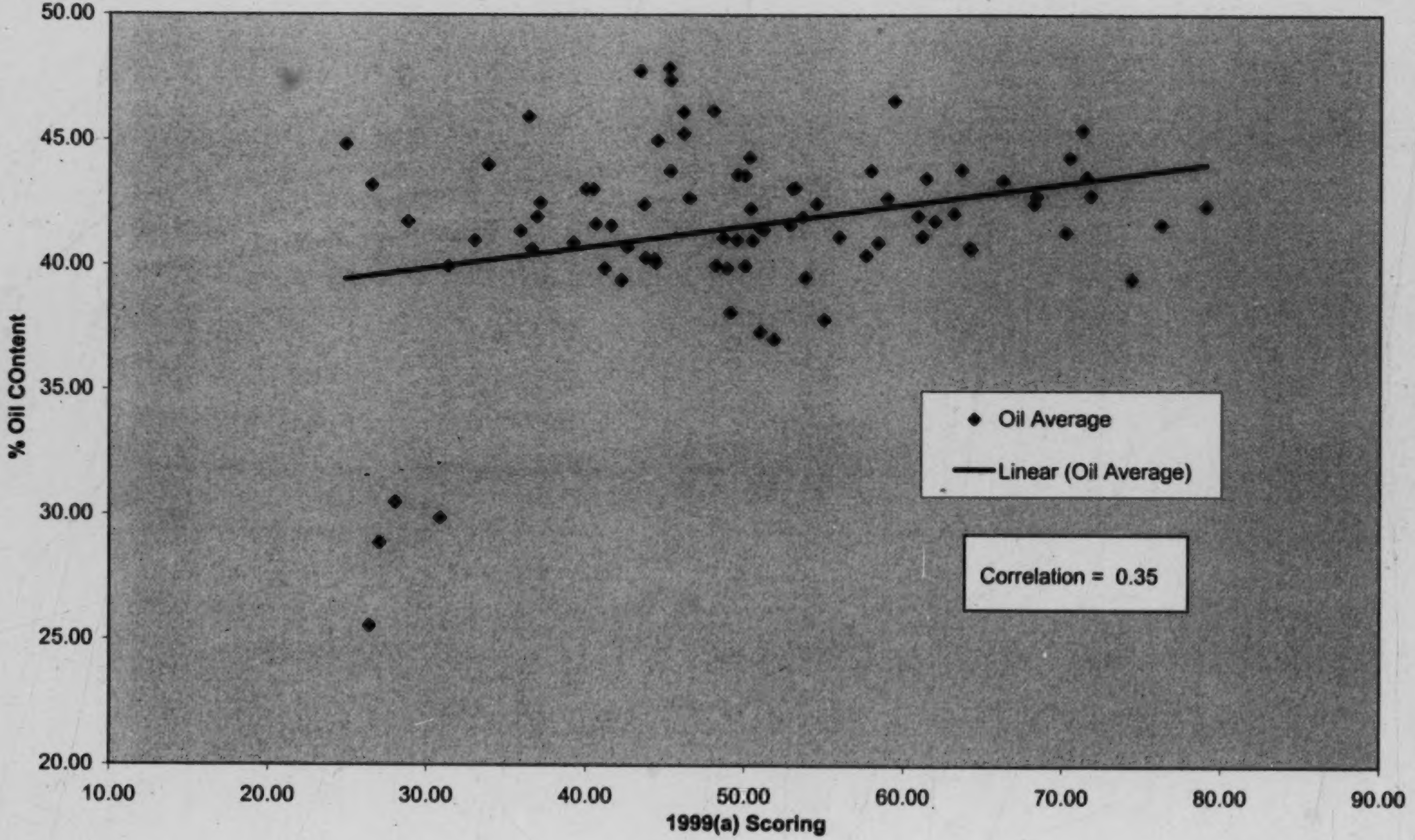
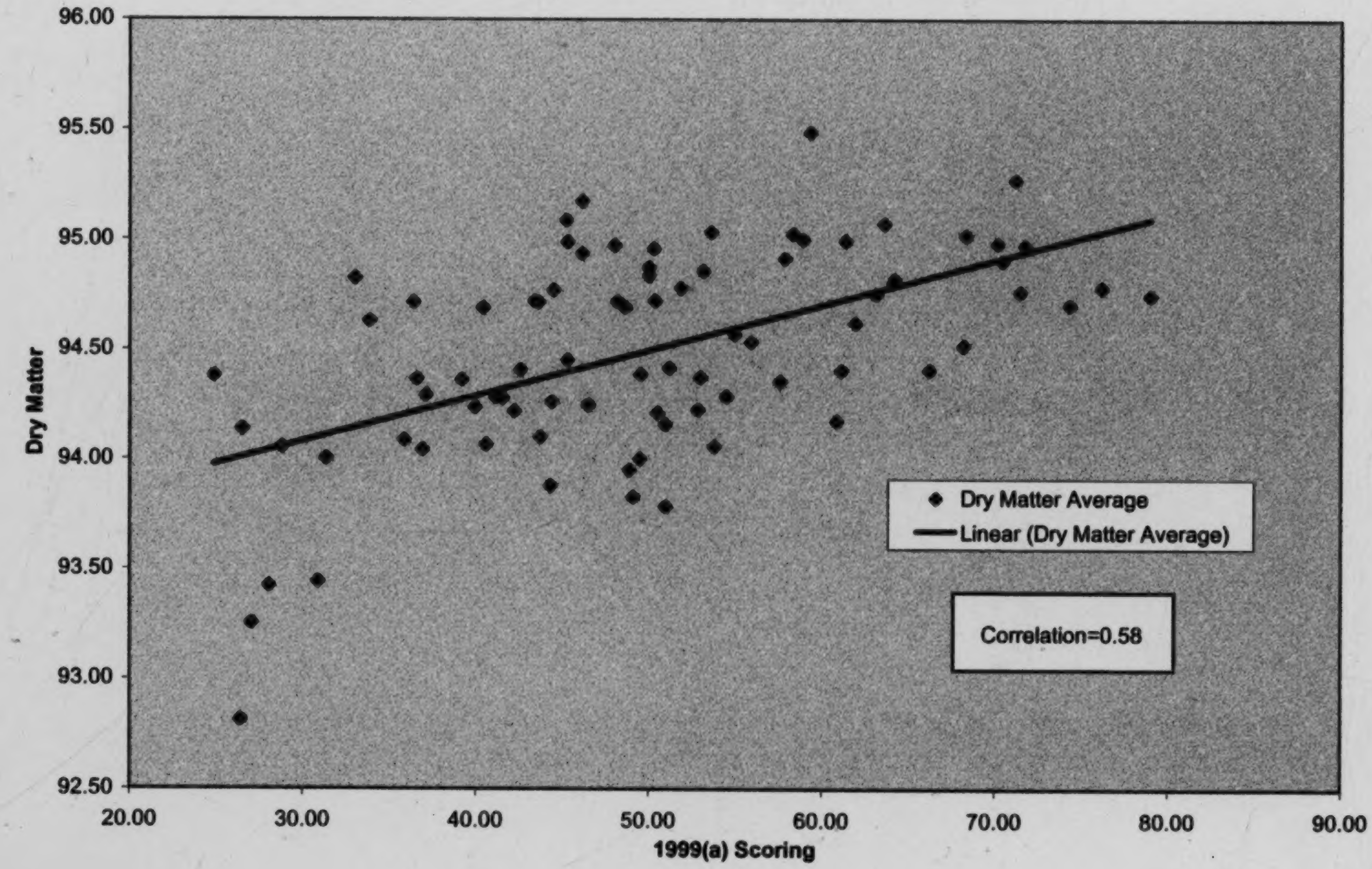


Figure 9: Scatter Plot of 1999(a) Flea Beetle Rating vs % Dry Matter



different (P<0.05) from resistant lines 98-B-4-176, 195 and 242. However, the remaining resistant lines, 98-B-4-107, 189, 243, 246, were not significantly different from 94-99 (Duncan's Multiple Test, P<0.05) and there was a negative correlation (r=-0.20) for 1999 (a) flea beetle rating to % protein.

Sinapis alba has a characteristically lower oil content and higher protein content than commercial *B. napus* (DeClercq and Daun, 2000) and this was evident in this testing as well. The S. *alba* lines (Sal-011, 016, 027, 044) had an average 31.1 % oil and 34.3 % protein whereas the entire 98-B-4 population and parentals had oil levels ranging from 37.0 % to 47.8 % and protein levels ranging from 23.5% to 31.8%. As evidenced by the industry data supplied in Table 4, the 2000 Canola oil industry average was 42.9% and yellow mustard industry average was 31.5%. The 2000 Canola protein industry average was 21.3% and the mustard industry average was 32.2%. Although lines 98- B-4-242, 243, 189, 176, 107, 195 and 246 had similar flea beetle damage ratings as *S. alba,* oil and protein profiles appear to be more similar to *B. napus*. Lines 98-B-4-242, 243, 246 had oil levels that met or exceeded Canola industry standards while all seven resistant 98-B-4 lines had protein contents which met or exceeded the canola quality average. The 1999 (a) flea beetle rating did positively correlate with % oil (r=0.35), % defatted protein (r=0.23) and % dry matter (r=0.58), although these correlations are low. Scatter plots have been provided for the correlations of early flea beetle rating to % oil (Figure 8) and % dry matter (Figure 9) to provide a clearer picture of the distribution of the data set.

3.3.3 Whole Seed Fatty Acid Content

Whole seed fatty acid profiles were determined for the 98-B-4 population, parental lines and several *S. alba* cultivars. Two samples were prepared for each individual and each sample was run in duplicate. The readings were then averaged to provide a mean reading for each line. A summary of the results is provided in Table 4 and the data set is provided in Appendix 6. Analysis of variance (Appendix 4) and Duncan's Multiple Range Test ($P<0.05$) were run on the raw whole seed fatty acid profile data. Significant differences ($P<0.05$) between the data observations for 94-99 (susceptible) and resistant individuals may suggest a relationship between flea beetle resistance and fatty acid components.

There was a significant difference ($P<0.05$) between the parental lines LK-001 and 94-99 for several of the fatty acids-including stearic, linolenic, gadoleic, behenic, erucic and total saturates. The parental line 94-99 is significantly different (Duncan's Multiple Range Test, $P<0.05$) from each of the seven flea beetle resistant lines for erucic acid (98-B-4-107, 176, 189, 195, 242, 243, 246) as well as for the three *S. alba* lines (Sal 11, 16, 27). The R-square value was 0.99 and the C.V. was 4.5 for the analysis of variance for erucic acid. However, the 1999 (a) rating does not correlate with erucic acid ($r= -0.26$). In addition, the three *S. alba* lines are significantly different from each other for erucic acid content ($P<0.05$) so there does not appear to be a relationship between flea beetle resistance and erucic acid.

Within the population, the lines demonstrate segregation for several components of the fatty acid profile. For example, lines 98-B-4-047, 174, 184 have a profile for oleic, linoleic, gadoleic and erucic acids which more closely resembles *S. alba* than their *B.*

Table 4: Summary of whole seed fatty acid profile analysis of the 98-B-4 DH mapping population. Duncan's Multiple Range Test ($P<0.05$) was used to separate the means. A different letter group indicates that the mean results are statistically different from one another.

Individual	1999(a)	1999(a)	C16:0	Duncan's	C16:1	Duncan's	C18:0	Duncan's	C18:1	Duncan's
	Scorings	Ratings	Palmitic	Multiple	Palmitoleic	Multiple	Stearic	Multiple	Oleic	Multiple
			%	Range	%	Range	%	Range	%	Range
				C16:0		C16:1		C18:0		C18:1
LK-001	49.09	N	4.29	A	0.32	A	1.84	A	58.24	A
94-99	50.95	N	4.46	A	0.28	A	1.52	B	59.39	A
98-B-4-242	24.87	R	4.05	A	0.22	B	1.45	B	56.16	A
98-B-4-243	26.50	R	4.37	A	0.26	A	1.64	B	58.61	A
98-B-4-189	28.80	R	4.09	A	0.33	A	1.36	C	57.98	A
98-B-4-176	31.34	R	4.04	A	0.29	A	1.24	C	54.44	B
98-B-4-107	33.01	R	4.37	A	0.32	A	1.41	B	56.20	B
98-B-4-246	33.84	R	4.00	A	0.25	A	1.56	B	60.48	A
98-B-4-195	35.86	R	4.60	A	0.30	A	1.44	B	55.02	B
98-B-4-187	36.39	N	4.15	A	0.25	A	1.42	B	58.47	A
98-B-4-178	36.58	R	4.28	A	0.36	A	1.55	B	55.94	B
98-B-4-190	36.91	N	3.80	B	0.23	B	1.56	B	56.54	AB
98-B-4-247	37.10	R	3.79	B	0.22	B	1.47	B	58.44	A
98-B-4-181	39.18	N	4.15	A	0.34	A	1.32	C	54.42	B
98-B-4-172	39.95	R	4.16	A	0.25	A	1.49	B	56.67	A
98-B-4-111	61.11	N	4.07	A	0.28	A	1.50	B	60.07	A
98-B-4-110	61.36	N	4.13	A	0.29	A	1.32	C	54.50	B
98-B-4-092	61.93	N	3.87	B	0.24	A	1.57	B	58.60	A
98-B-4-113	63.13	S	3.85	B	0.25	A	1.25	C	53.43	B
98-B-4-067	63.61	S	3.71	B	0.22	B	1.42	B	57.00	A
98-B-4-068	64.14	S	4.51	A	0.32	A	1.69	B	52.45	B
98-B-4-101	64.20	N	4.26	A	0.24	A	1.64	B	55.27	B
98-B-4-135	66.21	S	3.37	B	0.23	B	1.88	A	56.93	A
98-B-4-103	68.18	S	4.36	A	0.25	A	1.39	C	53.70	B
98-B-4-081	68.32	S	3.38	B	0.21	B	1.25	C	54.83	B
98-B-4-022	74.32	S	4.41	A	0.27	A	1.44	B	52.08	B
98-B-4-074	76.18	S	4.74	A	0.29	A	1.46	B	54.85	B
98-B-4-104	78.98	S	4.53	A	0.32	A	1.54	B	51.28	B
SAL-011 (*S. alba*)	26.41	R	2.12	D	0.20	B	0.73	D	25.33	C
SAL-016 (*S. alba*)	28.04	R	3.77	B	0.24	A	1.24	C	37.99	D
SAL-027 (*S. alba*)	30.87	R	2.53	C	0.19	B	1.04	E	29.41	E

Individual	1999(a) Scorings	1999(a) Ratings	C18:2 Linoleic %	Duncan's Multiple Range C18:2	C18:3 Linolenic %	Duncan's Multiple Range C18:3	C20:0 Arachidic %	Duncan's Multiple Range C20:0	C20:1 Gadoleic %	Duncan's Multiple Range C20:1	C20:2 Eicosadienoic %	Duncan's Multiple Range C20:2
LK-001	49.09	N	20.64	A	7.84	A	0.72	A	2.61	A	0.14	A
94-99	50.95	N	21.77	A	9.53	B	0.57	B	1.27	B	0.08	A
98-B-4-242	24.87	R	21.68	A	13.73	C	0.52	B	1.28	B	0.10	A
98-B-4-243	26.50	R	19.68	A	12.79	C	0.55	B	1.18	B	0.08	A
98-B-4-189	28.80	R	19.80	A	13.52	C	0.55	B	1.40	B	0.09	A
98-B-4-176	31.34	R	22.13	A	14.69	C	0.55	B	1.56	B	0.12	A
98-B-4-107	33.01	R	21.46	A	13.35	C	0.57	B	1.39	B	0.10	A
98-B-4-246	33.84	R	19.94	A	11.03	C	0.56	B	1.28	B	0.09	A
98-B-4-195	35.86	R	23.03	A	12.52	C	0.54	B	1.43	B	0.12	A
98-B-4-187	36.39	N	21.15	A	12.06	C	0.51	B	1.22	B	0.07	A
98-B-4-178	36.58	R	22.52	A	12.41	C	0.60	B	1.30	B	0.10	A
98-B-4-190	36.91	N	24.04	B	10.91	C	0.58	B	1.35	B	0.09	A
98-B-4-247	37.10	R	19.82	A	13.26	C	0.55	B	1.48	B	0.09	A
98-B-4-181	39.18	N	24.17	B	12.52	C	0.56	B	1.48	B	0.14	A
98-B-4-172	39.95	R	22.98	A	11.31	C	0.57	B	1.52	B	0.13	A
98-B-4-111	61.11	N	18.47	C	12.69	C	0.58	B	1.40	B	0.07	A
98-B-4-110	61.36	N	24.76	B	12.01	C	0.55	B	1.48	B	0.13	A
98-B-4-092	61.93	N	21.60	A	11.36	C	0.57	B	1.32	B	0.10	A
98-B-4-113	63.13	S	23.66	B	14.72	C	0.48	C	1.43	B	0.13	A
98-B-4-067	63.61	S	23.02	A	9.41	B	0.87	D	1.81	C	0.83	B
98-B-4-068	64.14	S	23.50	B	12.31	C	0.94	D	1.60	C	0.92	B
98-B-4-101	64.20	N	22.82	A	12.71	C	0.66	A	1.44	B	0.09	A
98-B-4-135	66.21	S	22.65	A	11.74	C	0.60	B	1.45	B	0.14	A
98-B-4-103	68.18	S	22.81	A	14.79	C	0.55	B	1.29	B	0.09	A
98-B-4-081	68.32	S	24.09	B	12.97	C	0.49	B	1.64	C	0.18	A
98-B-4-022	74.32	S	24.44	B	14.14	C	0.62	B	1.57	B	0.12	A
98-B-4-074	76.18	S	23.18	A	12.21	C	0.54	B	1.38	B	0.22	A
98-B-4-104	78.98	S	24.93	B	14.16	C	0.66	A	1.47	B	0.13	A
SAL-011 (*S. alba*)	26.41	R	9.96	D	11.68	C	0.52	B	8.04	D	0.28	A
SAL-016 (*S. alba*)	28.04	R	12.93	E	13.96	C	0.56	B	10.86	E	0.27	A
SAL-027 (*S. alba*)	30.87	R	10.44	D	12.03	C	0.62	B	9.96	F	0.32	C

Individual	1999(a)	1999(a)	C22:0	Duncan's	C22:1	Duncan's	C24:0	Duncan's	C24:1	Duncan's	Total	Duncan's
	Scorings	Ratings	Behenic	Multiple	Erucic	Multiple	Lignoceric	Multiple	Nervonic	Multiple	Saturates	Multiple
			%	Range	%	Range	%	Range	%	Range	%	Range
				C22:0		C22:1		C24:0		C24:1		Total Sats
LK-001	49.09	N	0.51	A	2.22	A	0.24	A	0.32	A	7.37	A
94-99	50.95	N	0.30	B	0.34	B	0.20	B	0.23	B	6.85	B
98-B-4-242	24.87	R	0.32	B	0.04	C	0.17	C	0.23	B	6.34	B
98-B-4-243	26.50	R	0.32	B	0.03	C	0.18	B	0.27	B	6.88	B
98-B-4-189	28.80	R	0.35	B	0.05	C	0.21	B	0.23	B	6.35	B
98-B-4-176	31.34	R	0.38	B	0.04	C	0.22	B	0.27	B	6.21	C
98-B-4-107	33.01	R	0.38	C	0.03	C	0.16	C	0.20	B	6.73	B
98-B-4-246	33.84	R	0.35	B	0.05	C	0.16	C	0.21	B	6.47	B
98-B-4-195	35.86	R	0.37	B	0.06	C	0.21	B	0.29	A	6.96	B
98-B-4-187	36.39	N	0.27	B	0.03	C	0.13	D	0.22	B	6.36	B
98-B-4-178	36.58	R	0.39	C	0.02	C	0.22	A	0.25	B	6.82	B
98-B-4-190	36.91	N	0.35	B	0.04	C	0.18	B	0.26	B	6.29	C
98-B-4-247	37.10	R	0.35	B	0.04	C	0.18	B	0.26	B	6.16	C
98-B-4-181	39.18	N	0.37	B	0.04	C	0.20	B	0.26	B	6.40	B
98-B-4-172	39.95	R	0.40	C	0.07	C	0.17	C	0.24	B	6.62	B
98-B-4-111	61.11	N	0.32	B	0.08	C	0.18	B	0.21	B	6.47	B
98-B-4-110	61.36	N	0.35	B	0.03	C	0.18	B	0.23	B	6.36	B
98-B-4-092	61.93	N	0.33	B	0.02	C	0.16	C	0.22	B	6.33	B
98-B-4-113	63.13	S	0.32	B	0.06	C	0.14	C	0.22	B	5.89	C
98-B-4-067	63.61	S	0.75	D	0.61	D	0.12	C	0.18	B	6.74	B
98-B-4-068	64.14	S	0.74	D	0.53	D	0.19	B	0.28	A	7.88	D
98-B-4-101	64.20	N	0.40	C	0.02	C	0.16	C	0.25	B	6.96	B
98-B-4-135	66.21	S	0.36	B	0.07	C	0.23	A	0.27	B	6.21	C
98-B-4-103	68.18	S	0.32	B	0.02	C	0.14	C	0.25	B	6.61	B
98-B-4-081	68.32	S	0.40	C	0.12	C	0.16	C	0.25	B	5.52	E
98-B-4-022	74.32	S	0.40	C	0.04	C	0.17	C	0.27	B	6.87	B
98-B-4-074	76.18	S	0.37	B	0.24	B	0.18	B	0.28	A	7.10	B
98-B-4-104	78.98	S	0.45	C	0.03	C	0.18	B	0.28	A	7.18	B
SAL-011 (*S. alba*)	26.41	R	0.54	A	37.23	E	0.28	E	3.06	C	3.90	F
SAL-016 (*S. alba*)	28.04	R	0.27	B	15.89	F	0.13	D	1.77	D	5.84	C
SAL-027 (*S. alba*)	30.87	R	0.47	A	30.36	G	0.22	A	2.34	E	4.66	G

napus parents. Two of these lines 98-B-4-174 and 184 also show a slight improvement in flea beetle antixenosis from LK-001 and 94-99 at readings of 41.55 and 45.29 so there may be a relationship of the fatty acid profile with flea beetle resistance.

Erucic acid is the main fatty acid component in the oils of mature seeds of *S. alba*. However, in the mature plant tissues there are only trace amounts as compared to cotyledons aged 4 to 8 days (Kumar and Tsunoda, 1980). Linolenic acid is low in *S. alba* seeds but increases as the seed germinates and develops (Liu and Brown, 1996). As the *S. alba* seed develops into a plant and tissues differentiate, the fatty acid profile changes from the profile seen in the original seed (Yaniv *et al.*, 1998). This suggests that an examination of whole seed fatty acids may not provide useful information for insect pest resistance in the growing plant. It may be more informative to study the *S. alba* fatty acid profile in the cotyledon stage as this is when the plant is most vulnerable and this may apply to the 98-B-4 population as well.

3.3.4 Half Seed Fatty Acid Content

Half seed fatty acid analysis was conducted on a subset of the 98-B-4 population (Table 5). Within this limited data set there is a strong correlation for the 1999 (a) flea beetle rating to palmitic acid (r=0.77, Figure 10), palmitoleic (r=0.77, Figure 11), linoleic (r=0.74, Figure 12), behenic (r=0.62, Figure 13) and total saturates (r=0.76, Figure 14). Additionally, other fatty acid components have positive correlations with the early flea beetle rating-stearic (r=0.59) and arachidic (r=0.59). These correlations would suggest that cotyledon fatty acid components may play a role in the level of flea beetle antixenosis of oilseed crops.

As stated earlier, in *S. alba* seedlings the fatty acid profile is different from the profile seen in the original seed (Yaniv *et al.*, 1998). For example, in this data the cotyledon palmitoleic acid level in Sal-016 was 0.12 (Table 5) whereas the whole seed level was 0.24 (Table 4). For LK-001 the cotyledon palmitoleic level was 0.13 (Table 5) and the seed level was 0.32 (Table 4). A similar trend was seen in the 98-B-4 population lines that were rated as resistant (98-B-4-176, 189, 242 and 243). Oilseeds are most vulnerable to flea beetle attack and damage at the cotyledon stage. The data presented here suggests that perhaps the levels of specific fatty acid components within the cotyledon could assist in the selection of flea beetle resistant lines.

Table 6: Mean half – seed cotyledon fatty acid profile analysis for selected lines from the 98-B-4 DH mapping population.

Individual	1999(a)	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	Total
	Scoring	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Arachidic	Gadoleic	Eicosadienoic	Behenic	Erucic	Lignoceric	Nervonic	Saturates
		%	%	%	%	%	%	%	%	%	%	%	%	%	%
LK-001	49.09	3.38	0.13	1.14	65.78	17.33	9.30	0.47	1.49	0.08	0.34	0.06	0.25	0.21	5.32
94-99	50.89	4.07	0.21	1.38	63.19	18.17	11.10	0.46	0.85	0.06	0.24	0.01	0.08	0.14	6.14
98-B-4-022	74.32	6.95	0.44	2.08	39.43	32.75	13.88	1.02	1.81	0.17	1.06	0.01	0.15	0.25	11.11
98-B-4-074	76.18	5.58	0.20	1.71	51.66	25.55	12.34	0.68	1.19	0.14	0.41	0.00	0.20	0.29	8.38
98-B-4-099	48.01	3.69	0.16	1.37	58.25	23.35	10.65	0.53	1.20	0.09	0.30	0.00	0.16	0.22	5.89
98-B-4-104	78.98	6.19	0.40	2.07	41.52	30.80	12.50	1.05	1.55	0.00	1.28	0.00	1.46	1.18	10.60
98-B-4-126	52.83	5.12	0.20	1.26	45.86	31.46	12.23	0.61	1.42	0.17	0.51	0.52	0.19	0.39	7.49
98-B-4-176	31.34	4.67	0.19	0.98	54.76	24.10	12.15	0.49	1.54	0.18	0.40	0.00	0.24	0.24	6.53
98-B-4-189	28.80	4.09	0.14	1.51	55.14	20.91	15.45	0.60	1.15	0.11	0.39	0.00	0.20	0.25	6.60
98-B-4-242	24.87	4.02	0.15	1.59	61.64	19.65	10.71	0.59	0.97	0.09	0.31	0.00	0.08	0.16	6.50
98-B-4-243	26.50	4.00	0.08	1.87	67.57	15.62	7.39	0.85	1.40	0.10	0.54	0.00	0.29	0.27	7.26
BNA-038 (*B.napus*)	39.33	3.75	0.16	1.29	67.55	16.65	8.29	0.48	1.15	0.06	0.28	0.04	0.15	0.11	5.80
Sal-016 (*S.alba*)	28.04	4.09	0.12	0.92	36.60	13.52	15.92	0.42	9.81	0.26	0.25	15.96	0.22	1.79	5.68
Sal-044 (*S.alba*)	27.04	2.33	0.17	0.76	27.94	8.52	13.23	0.51	8.59	0.24	0.53	33.77	0.24	2.07	4.12
Correlation to 1999 (a) scoring		0.77	0.77	0.59	-0.19	0.74	0.08	0.59	-0.35	-0.34	0.62	-0.37	0.45	-0.15	0.76

Figure 10: Scatter Plot of 1999(a) Flea Beetle Rating vs. C16:0 Palmitic (Half Seed)

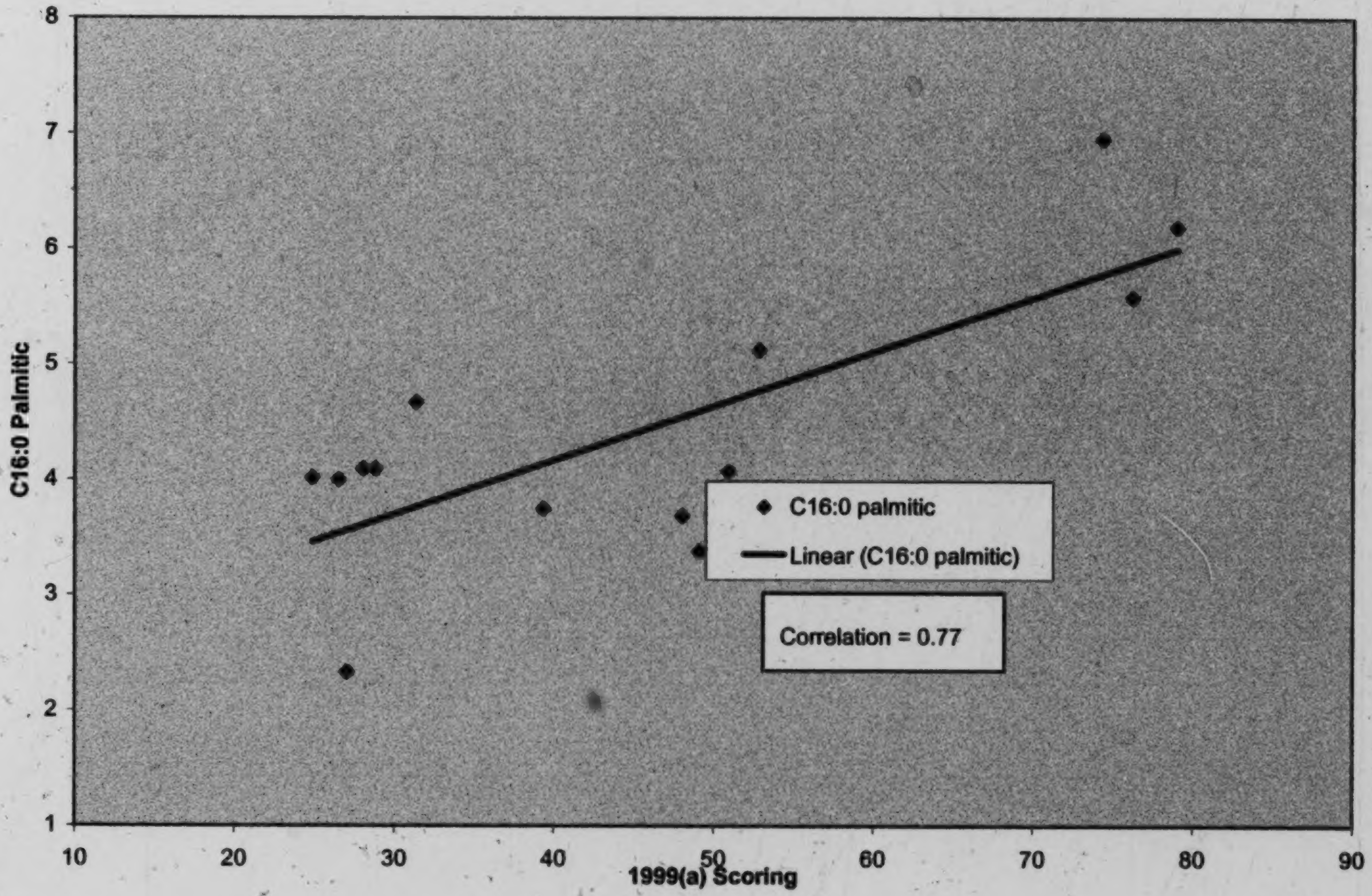


Figure 11: Scatter Plot of 1999(a) Flea Beetle Rating vs. C16:1 Palmitoleic (Half Seed)

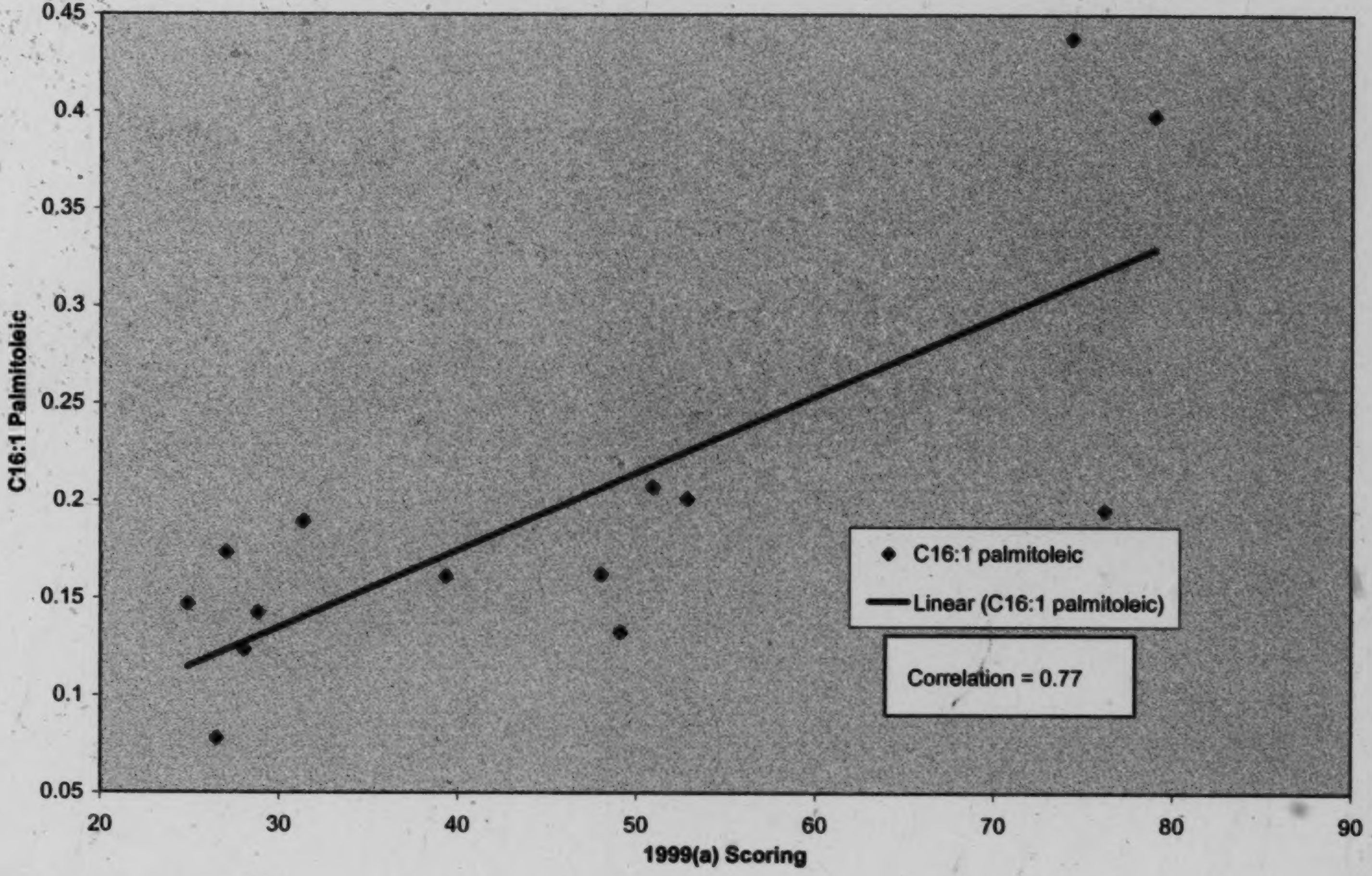


Figure 12: Scatter Plot of 1999(a) Flea Beetle Rating vs. C18:2 Linoleic (Half Seed)

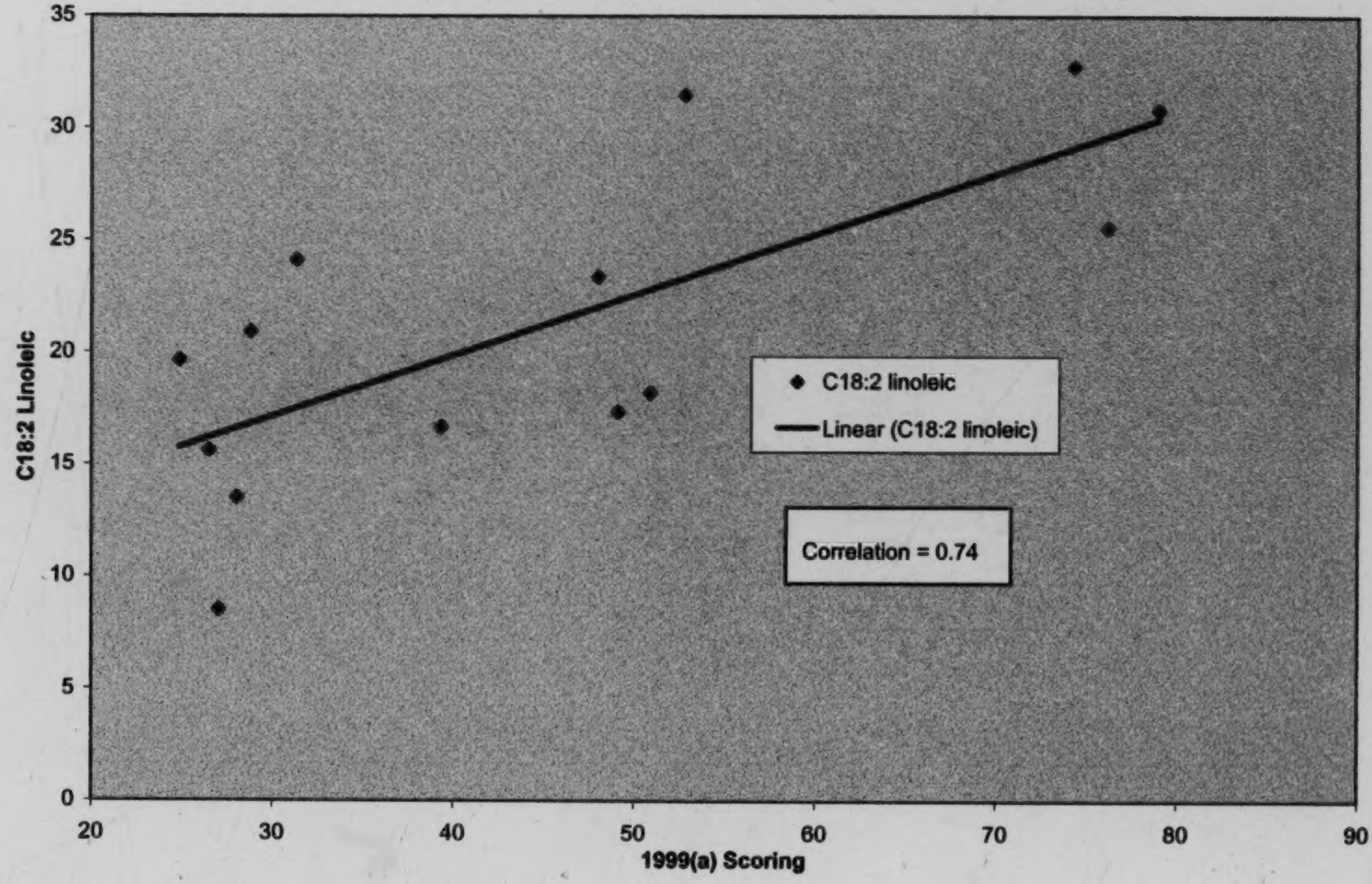


Figure 13: Scatter Plot of 1999(a) Flea Beetle Rating vs. C22:0 Behenic (Half Seed)

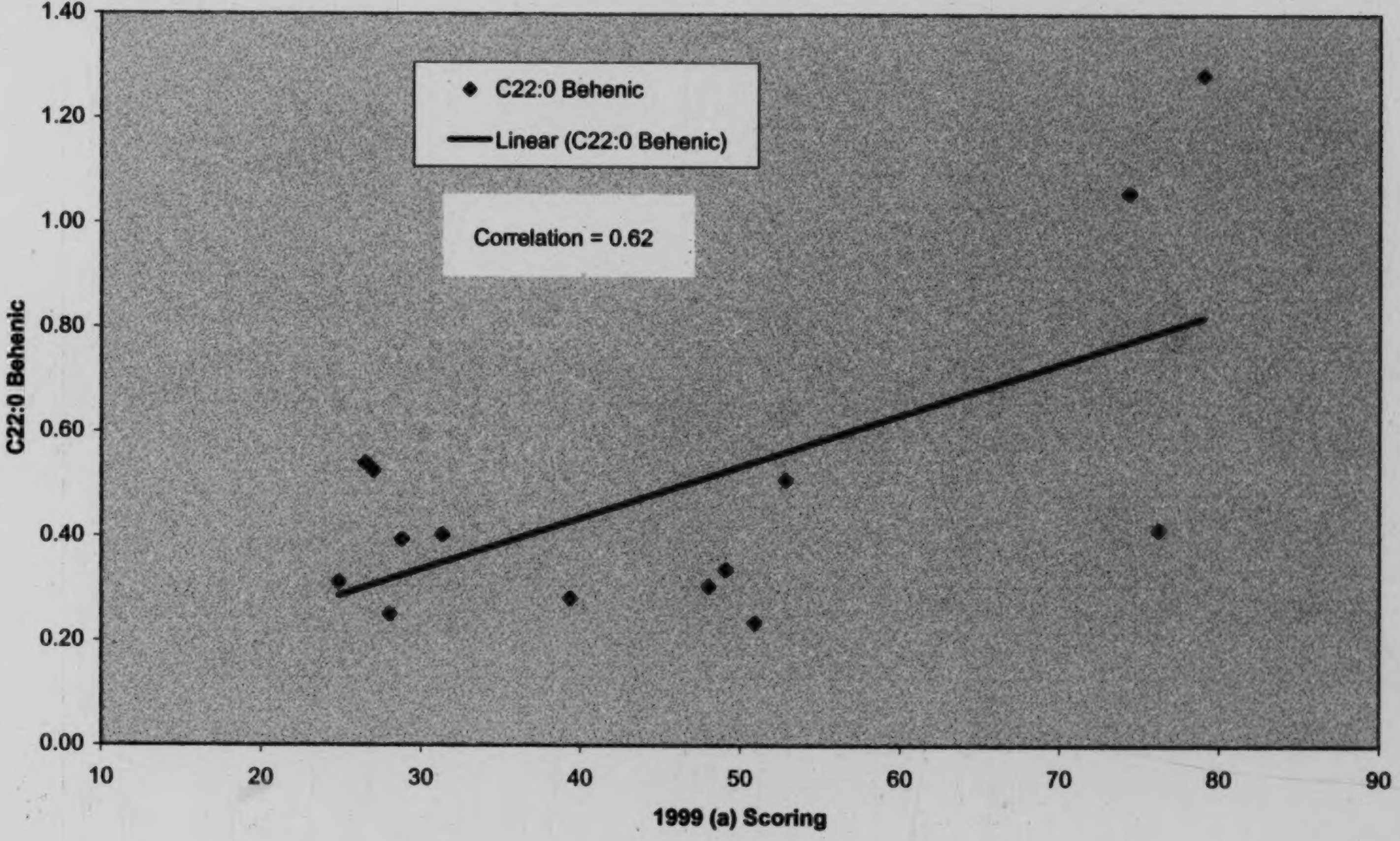
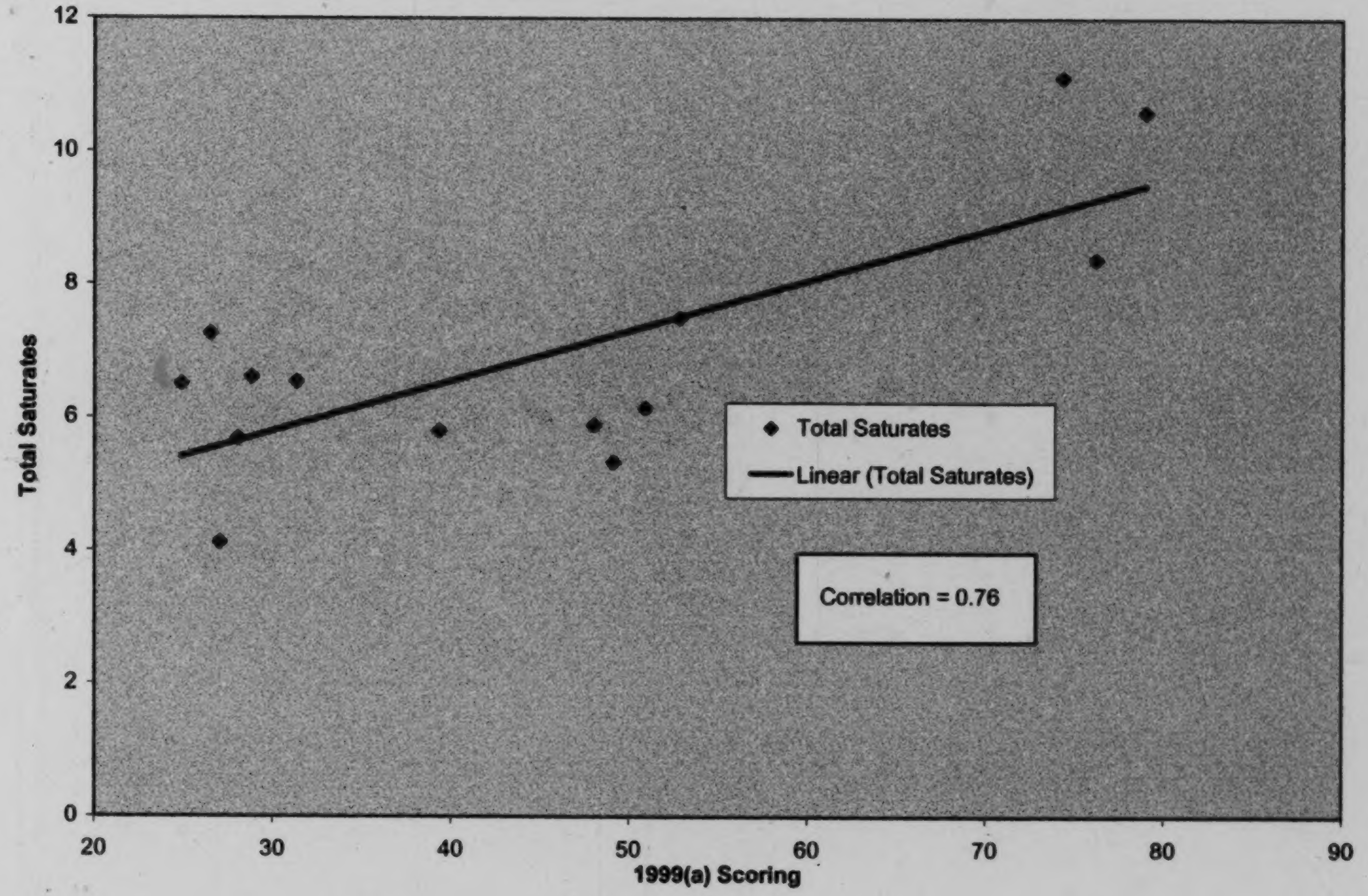


Figure 14: Scatter Plot of 1999(a) Flea Beetle Rating vs. Total Saturates (Half Seed)



IV. Conclusions

Flea beetle damage to canola seedlings causes significant economic hardship to farmers across the Prairies, the United States and in Europe. Insecticides applied to protect crops are costly, pose both environmental and health hazards, and does not ensure complete control of the pest. As a result, plant breeders have long searched for viable means to introduce host plant pest resistance in canola.

The work presented within this thesis provides the first known attempt at developing an RFLP linkage map for flea beetle resistance in *B. napus*. Initial analysis of the mapping data has placed a putative flea beetle resistance QTL on linkage group two, in the region spanning molecular markers pec2e4, pec2h2b, pec5e12 and pec3a8c. The linkage groups proposed here will provide a basis for other researchers to map the gene (s) for flea beetle resistance while developing new canola varieties. The completion of such a map could provide new tools for the development of novel insect resistant canola cultivars. Plant breeders evaluate thousands of lines annually in order to select for potential new varietal candidates. The RFLP map we developed would allow researchers to screen for insect resistance using a molecular approach as opposed to field studies carried out over several years. By performing RFLP or molecular analysis of the lines of interest, a researcher can identify the presence or absence of the alleles that confer flea beetle resistance to an individual plant and decide quickly which lines to move ahead in the breeding program. This aspect could save years in the development of new commercial cultivars.

We developed a double haploid population of *Brassica napus* plants that segregate for flea beetle resistance. One of the major difficulties with this study is that the population was studied and evaluated for only a single year, and the protocol involved using an indoor laboratory procedure. Supplementary and confirming studies should be conducted both in the laboratory and in the field. Multiple rounds of testing are advisable to increase confidence in the results and to ensure that accurate scoring of the different doubled haploid lines. Ultimately, these lines or genotypes derived from these lines will need to perform in a producer's field in various growing conditions, so subsequent evaluations should be carried out in this environment.

The development of the flea beetle mapping population offers an opportunity for additional studies of patterns of flea beetle attack. This thesis concentrated on the role of seed quality parameters in relationship to flea beetle resistance and indicates that resistance is not correlated with a number of traits such as total glucosinolate levels and seed fatty acid components. This supports previous work of Åhman (1982) and Rawlinson and Williams (1991) who also found no correlation between resistance and levels of aliphatic glucosinolates. Our work does not explore physical aspects of the plant and the possible impact they may play in deterring flea beetles from attacking the canola plants. However, the development of the mapping populations provides an opportunity for studies of flea beetle behavior and patterns of attack. Adult flea beetles feed on cotyledons and young stems of seedlings and continue to attack the leaves as the plant develops (Feeney *et al.*, 1970). The segregating population could allow researchers to examine the role of trichomes, leaf shape and arrangements, and seedling growth patterns on the mode of flea beetle attack., providing there was genetic variation for these traits that could be reliably measured. This would include studying physical characteristics that attract flea beetles and additional characteristics that may determine if the flea beetle will stay and feed, or leave to search for other favorable hosts. Comparisons of the genetic components that may lead flea beetles to attack susceptible plants and to ignore resistant plants could be conducted.

Whole seed analysis data provided evidence that flea beetle resistant *B. napus* cultivars of canola quality may be possible. However in addition to having a whole seed canola quality profile, varieties must also meet certain yield performance levels; disease resistance levels and adapt to a broad range of production areas. The parental line LK-001, although flea beetle resistant, was lacking in several of these critical areas. The populations developed for this thesis should be evaluated in replicated trials for their seed yielding ability, disease resistance and the stability of their seed quality components. In addition to a completion of the 98-B-4 population linkage map, a study of the segregation of the seed and seedling quality traits with RFLP markers should be conducted. Oil, protein and dry matter whole seed analysis show some positive correlation with flea beetle resistance. The initial data set of one year, one location is quite limited but does provide an interesting starting point for further analysis. This information could provide more definitive answers to a possible relationship between flea beetle resistance and oil, protein and dry matter parameters. There is the opportunity to expand the linkage map developed here to map these quality traits when additional analyses using MAPMAKER/QTL are carried out.

Glucosinolates have long been suggested to have a role in insect resistances. To date no studies have shown a conclusive relationship between flea beetle resistance and total glucosinolate content. Total seed glucosinolates are comprised of several individual and specific constituents. The whole seed glucosinolate data contributed by this thesis show a positive correlation for flea beetle resistance and total indolyl glucosinolates and 4-hydroxy-3-indolylmethyl glucosinolate. A more detailed study of these specific components, their placement in the glucosinolate pathway and their role in the canola seed may be warranted. It may be that individual glucosinolate components are more directly related to flea beetle resistance as opposed to total glucosinolate levels.

Flea beetle attack canola seedlings when they are in the cotyledon stage. The whole seed fatty acid profiles of the mapping population show small positive correlations for specific fatty acid components, however the strongest correlations were seen in the half-seed cotyledon analysis. Our limited data set implies that the fatty acid profile in seedling cotyledons may play a role in the ability of a crop seedling to resist flea beetle attack. Positive correlations with flea beetle resistance were in evidence for palmitic, palmitoleic, linoleic, behenic fatty acids and total saturates for the half-seed analysis. Cotyledon analysis was conducted on a small portion of the mapping population, based on one location, one year of production seed. An analysis of the complete 98-B-4 mapping population for half-seed fatty acid components would be an important continuation of this work. Additional locations and replicates of the mapping population seed should be grown and tested. In particular an in-depth examination of the fatty acid profiles and the enzymes involved in these pathways may provide insight into a possible relationship between individual fatty acid components and their role on flea beetle resistance. The study of half seed cotyledon analysis may provide a more conclusive answer to the role, if any really exists, of specific fatty acid components in the insect resistance of *S. alba*.

This research identified genetic sources of flea beetle resistance within a hybrid *B. napus/S. alba* population and began to determine the genetic basis of this resistance using molecular markers. Specifically, a double haploid (DH) population of *B. napus* plants, which segregated for flea beetle resistance, was developed from a cross between a resistant hybrid and susceptible *B. napus*. This thesis provides the framework of the first known RFLP linkage map for flea beetle resistance in *B. napus*. Flea beetle damage rating and seed quality analysis (oil, defatted protein, glucosinolate and fatty acid levels) were determined for the parental lines and the DH population. A preliminary study of the relationships between flea beetle ratings and seed parameters were conducted. Our initial data suggests that seed and seedling characteristics may play a role in the flea beetle resistance of canola cultivars.

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VI. Appendices

Appendix 1: Multi-year testing data for the parentals and related lines of the 98-A-4 and 98-B-4 DH population. (Gavloski *et al*., 2000)

Line	1995	1996	1997	1998(1)	1998(2)	1998(3)
LKO-001	**R+**	**R**	**R**	**R**	**R**	
94-99 (BNA-084)				**S**	**N**	**N**
LKO-009	R+	N		N	N	
LKO-010	R	N		N	N	
LKO-029		N	R	N		
LKO-032	R	N			N	
LKO-049			R	S	N	
LKO-052		N	N			
LKO-055	R	N		N	N	
LKO-062			S	N		
LKO-063		N	N			
LKO-064		N	N			
LKO-071		S		N		
LKO-072			N			
LKO-089			R	N		
LKO-101		S		N	N	
LKO-106			R		N	
LKO-124	R+	N		N		
LKO-127		S	S	S	N	
LKO-130		N	S			
LKO-133		N	R	N	N	N
LKO-134	R	R		N	N	
LKO-158		N	R	N	S	R
LKO-181			R	S	N	
LKO-182		S	N			
LKO-192			R	N	N	
LKO-277	R	N			S	N
LKO-288		N		N	N	N
LKO-289			N			
LKO-296		R	N	S	N	
LKO-336			S	N	N	
LKO-338		S		N	N	
LKO-340			R	S	N	
LKO-365			R	N	N	
LKO-371			R	S	N	
LKO-398			R		N	
LKO-401		N	R	N		
LKO-413			R		N	N
LKO-418			N			
LKO-420		N	N			
LKO-424		S				
LKO-426		S	N			
LKO-427		S				
LKO-429	R	N				
LKO-550	R+	N		S	N	
LKO-569		**R**	**R**	**N**	**R**	

Appendix 2: Summary of allelic frequency in the 98-B-4 Dh mapping populaiton. n+=LK-001 allele, n-=94-99 allele, n(0)=null individual. "*", "**", "***", "****" designate significant distortions.

Linkage Group	Probe	n+	n-	n(0)	%n-	X2	P	
1	*pwg4d7	38	39	3	50.6	0.01	0.91	
1	*pwg6a12	40	35	5	46.7	0.33	0.56	
1	*pec4c11	41	34	5	45.3	0.65	0.42	
2	*pec3e12a	44	23	13	34.3	6.58	0.01	*
2	*pec3h4	34	41	5	54.7	0.65	0.42	
2	*ptg2b4	31	44	5	58.7	2.25	0.13	
2	*pec2e4	33	34	13	50.7	0.01	0.90	
2	*pec2h2b	33	45	2	57.7	1.85	0.17	
2	*pec5e12	31	47	2	60.3	3.28	0.07	
2	*pec3a8c	23	54	3	70.1	12.48	0.00	***
3	*ptg2f12	35	41	4	53.9	0.47	0.49	
3	*ptg2h10	38	39	3	50.6	0.01	0.91	
3	*pwg1a10a	37	37	6	50.0	0.00	1.00	
3	*pwg5a5	33	37	10	52.9	0.23	0.63	
4	*ptg1h12a	45	34	1	43.0	1.53	0.22	
4	*pec2c7	42	36	2	46.2	0.46	0.50	
4	*pwg3h8	39	35	6	47.3	0.22	0.64	
4	*pwg4h5	41	36	3	46.8	0.32	0.57	
5	*ptg5h12	35	38	7	52.1	0.12	0.73	
5	*pwg2c1	32	45	3	58.4	2.19	0.14	
5	*pwg2a3b	20	57	3	74.0	17.79	0.00	***
6	*pwg3c9b	28	46	6	62.2	4.38	0.04	*
6	*pwg1a4e	31	44	5	58.7	2.25	0.13	
6	*pwg3h6	30	34	16	53.1	0.25	0.62	
7	*pec4h9b	43	32	5	42.7	1.61	0.20	
7	*pec4h3	44	33	3	42.9	1.57	0.21	
7	*pwg1g10b	44	32	4	42.1	1.89	0.17	
7	*pec4f11	37	37	6	50.0	0.00	1.00	
7	*ptg5d9a	44	32	4	42.1	1.89	0.17	
8	*pwg7b6	36	35	9	49.3	0.01	0.91	
8	*pec4g4	36	38	6	51.4	0.05	0.82	
8	*pwg2e12	43	32	5	42.7	1.61	0.20	
9	*pwg1h5	37	37	6	50.0	0.00	1.00	
9	*ptg4d2a	47	31	2	39.7	3.28	0.07	
9	*pec4h9a	47	25	8	34.7	6.72	0.01	*
9	*pec6b2	38	29	13	43.3	1.21	0.27	
10	*pwg7b3	42	35	3	45.5	0.64	0.43	
10	*pec3f1b	44	29	7	39.7	3.08	0.08	
10	*pec3g3b	44	28	8	38.9	3.56	0.06	
10	*pwg3f7	48	26	6	35.1	6.54	0.01	*
11	*pwglg5a	40	39	1	49.4	0.01	0.91	
11	*pec4h7	33	42	5	56.0	1.08	0.30	
11	*ptg6c3	24	52	4	68.4	10.32	0.00	***
11	*pec3b4aNM	19	53	8	73.6	16.05	0.00	***
12	*pwg1e3	46	32	2	41.0	2.51	0.11	
12	*pec5c4c	50	23	7	31.5	9.99	0.00	***
12	*pwg2g9a	68	4	8	5.6	5000.00	0.00	***

Appendix 2: Summary of allelic frequency in the 98-B-4 Dh mapping populaiton. n+=LK-001 allele, n-=94-99 allele, n(0)=null individual. "*", "**", "***", "****" designate significant distortions.

Linkage Group	Probe	n+	n-	n(0)	%n-	X2	P	
13	*pec5c4b	36	34	10	48.6	0.06	0.81	
13	*pwg1a4c	41	34	5	45.3	0.65	0.42	
13	*pec3d2b	42	29	9	40.8	2.38	0.12	
14	*pec2e5a	55	22	3	28.6	14.14	0.00	***
14	*pwg3h4	56	23	1	29.1	13.78	0.00	***
14	*pwg8h5a	55	24	1	30.4	12.16	0.00	***
14	*pwg1g10a	56	18	6	24.3	19.50	0.00	***
15	*pec2d1c	35	37	8	51.4	0.06	0.81	
15	*pec3f1a	53	23	4	30.3	11.84	0.00	***
15	*pwg1a4a	51	23	6	31.1	10.59	0.00	***
16	*pwg2a3a	18	61	1	77.2	23.08	0.00	***
16	*pec2h2a	3	71	6	95.9	5000.00	0.00	***
16	*ptg4d2b	22	55	3	71.4	14.14	0.00	***
17	*pec2d1b	27	40	13	59.7	2.52	0.11	
17	*pec3g3c	42	29	9	40.8	2.38	0.12	
17	*pwg1g2	41	31	8	43.1	1.39	0.24	
18	*pec3a8	29	37	14	56.1	0.97	0.32	
18	*pwg1a4d	18	47	15	72.3	12.94	0.00	***
18	*pec3b4cNP	30	47	3	61.0	3.75	0.05	
Unlinked	*pwg1a10b	33	44	3	57.1	1.57	0.21	
Unlinked	*pec2d1a	27	46	7	63.0	4.95	0.03	*
Unlinked	*pec3d2a	40	31	9	43.7	1.14	0.29	
Unlinked	*pec3b2	29	32	19	52.5	0.15	0.70	
Unlinked	*pec3e12b	30	44	6	59.5	2.65	0.10	
Unlinked	*pwg1a4b	36	25	19	41.0	1.98	0.16	
Unlinked	*pwg8h5b	36	43	1	54.4	0.62	0.43	
Unlinked	*pec2d8	51	24	5	32.0	9.72	0.00	**
Unlinked	*ptg5d9b	44	33	3	42.9	1.57	0.21	
Unlinked	*pec5c4d	33	41	6	55.4	0.86	0.35	
Unlinked	*pwg9e9	41	37	2	47.4	0.21	0.65	
Unlinked	*pec2c12	34	30	16	46.9	0.25	0.62	
Unlinked	*pec2e12	33	37	10	52.9	0.23	0.63	
Unlinked	*pwg6h1	47	31	2	39.7	3.28	0.07	
Unlinked	*pwg3c9a	68	4	8	5.6	5000.00	0.00	***
Unlinked	*pec5c4aNP	41	33	6	44.6	0.86	0.35	
Unlinked	*pwg2g9b	40	33	7	45.2	0.67	0.41	
Unlinked	*pec3a8b	44	32	4	42.1	1.89	0.17	
Unlinked	*pwg2a3c	34	41	5	54.7	0.65	0.42	
Unlinked	*pwg8h5c	14	65	1	82.3	5000.00	0.00	***
Unlinked	*pec3b4b	44	27	9	38.0	4.07	0.04	*
Unlinked	*pwg3g11	41	37	2	47.4	0.21	0.65	
Unlinked	*pec2e5b	33	43	4	56.6	1.32	0.25	
Unlinked	*pec3b12	34	42	4	55.3	0.84	0.36	
Unlinked	*pwg7d9	34	41	5	54.7	0.65	0.42	
Unlinked	*pec3g3a	26	46	8	63.89	5.56	0.02	*

Appendix 3: Mean glucosinolate profile data for the 98-B-4 DH mapping population.

Individual	1999(a)	1999(a)	Total	Total	Total	Total	3-Butenyl	4-Pentenyl	2-Hydroxy	2-Hydroxy	3-Indolylmethyl	4-Hydroxy
	Scorings	Ratings	Alkenyl	Indolyl	Glucosinolate	Allyl			3-Butenyl	4-Pentenyl		3-Indolylmethyl
			um/g	um/g	um/g oil free meal	um/g	um/g	um/g	um/g	um/g	um/g	um/g
LK-001	49.09	N	8.70	13.51	22.21	0.10	2.55	0.69	5.15	0.20	1.93	11.58
94-99	50.95	N	7.89	9.42	17.31	0.19	1.55	0.53	5.18	0.45	1.19	8.23
98-B-4-242	24.87	R	10.96	11.77	22.73	0.14	3.08	1.27	6.05	0.42	0.57	11.20
98-B-4-243	26.50	R	16.76	11.26	28.02	0.12	4.43	3.14	8.24	0.83	1.11	10.15
98-B-4-189	28.80	R	6.91	11.23	18.15	0.05	2.19	0.83	3.63	0.21	0.83	10.40
98-B-4-176	31.34	R	5.56	13.17	18.73	0.00	1.91	0.72	2.78	0.13	1.92	11.25
98-B-4-107	33.01	R	18.38	9.98	28.36	0.12	3.21	2.22	11.07	1.77	0.52	9.47
98-B-4-246	33.84	R	15.24	6.65	21.89	0.14	2.82	2.33	8.39	1.57	0.28	6.37
98-B-4-195	35.86	R	7.48	7.66	15.14	0.08	2.49	1.16	3.38	0.37	0.51	7.15
98-B-4-187	36.39	N	5.25	6.57	11.83	0.11	1.14	0.43	3.37	0.20	0.20	6.38
98-B-4-178	36.58	R	10.12	7.18	17.30	0.04	2.34	1.26	6.02	0.46	0.29	6.89
98-B-4-190	36.91	N	11.59	11.08	22.67	0.13	2.07	0.67	8.07	0.65	2.24	8.84
98-B-4-247	37.10	R	10.61	7.90	18.51	0.15	2.75	1.29	5.76	0.67	0.34	7.56
98-B-4-181	39.18	N	5.14	5.40	10.54	0.00	1.38	0.72	2.88	0.16	0.26	5.14
98-B-4-172	39.95	R	6.56	13.82	20.38	0.11	1.44	0.38	4.37	0.26	0.48	13.34
98-B-4-064	40.41	N	8.77	16.11	24.88	0.00	2.65	0.77	5.14	0.21	2.64	13.48
98-B-4-194	40.58	N	11.06	10.76	21.82	0.04	3.52	1.41	5.70	0.39	0.33	10.43
98-B-4-180	41.13	N	8.29	5.61	13.90	0.02	1.67	0.56	5.64	0.40	0.50	5.11
98-B-4-174	41.55	N	24.19	11.32	35.50	0.08	4.43	1.91	16.63	1.14	1.47	9.85
98-B-4-241	42.20	N	6.27	11.66	17.93	0.07	1.82	0.90	3.17	0.30	1.18	10.48
98-B-4-116	42.58	N	9.98	10.70	20.68	0.19	2.56	1.47	4.91	0.84	0.61	10.09
98-B-4-157	43.38	N	5.03	10.58	15.62	0.00	1.16	0.40	3.25	0.23	0.50	10.09
98-B-4-245	43.62	N	2.33	4.48	6.81	0.06	0.67	0.37	1.09	0.14	0.17	4.32
98-B-4-179	43.72	N	4.08	8.14	12.21	0.04	1.10	0.21	2.67	0.06	0.20	7.94
98-B-4-133	44.29	N	18.20	15.28	33.48	0.08	14.51	2.09	1.42	0.08	2.54	12.74
98-B-4-182	44.37	N	4.69	7.19	11.88	0.06	1.64	0.48	2.41	0.10	0.27	6.93
98-B-4-003	44.48	N	6.76	16.10	22.85	0.00	2.26	0.62	3.61	0.28	2.13	13.97
98-B-4-017	45.21	N	4.92	12.06	16.98	0.02	2.10	0.32	2.44	0.05	0.52	11.53
98-B-4-192	45.29	N	3.27	8.19	11.45	0.12	0.80	0.24	1.95	0.15	0.81	7.38
98-B-4-184	45.29	N	7.84	9.81	17.65	0.06	2.39	0.56	4.49	0.35	0.57	9.24
98-B-4-004	46.13	N	4.38	7.70	12.08	0.00	0.87	0.30	3.09	0.12	0.38	7.32
98-B-4-014	46.13	N	8.46	10.40	18.86	0.00	2.21	1.08	5.06	0.12	0.57	9.83
98-B-4-244	46.48	N	8.57	9.01	17.57	0.05	1.37	0.71	5.66	0.79	0.19	8.82
98-B-4-099	48.01	N	13.92	14.51	28.43	0.10	3.54	1.21	8.70	0.37	1.11	13.40
98-B-4-070	48.17	N	7.42	11.79	19.21	0.00	1.02	0.46	5.60	0.34	1.11	10.68
98-B-4-009	48.62	N	12.87	12.96	25.84	0.00	2.28	0.74	9.08	0.77	2.26	10.70
98-B-4-173	48.86	N	15.84	13.04	28.88	0.11	5.75	0.98	8.70	0.30	0.72	12.33
98-B-4-191	49.48	N	7.34	10.48	17.82	0.20	2.90	0.69	3.36	0.20	0.55	9.93
98-B-4-185	49.52	N	2.41	7.47	9.88	0.11	0.67	0.16	1.35	0.11	0.26	7.22
98-B-4-073	50.00	N	9.63	10.46	20.08	0.06	2.85	1.28	4.97	0.46	0.48	9.98
98-B-4-063	50.00	N	12.28	12.07	24.35	0.05	2.72	1.66	7.14	0.72	1.30	10.77
98-B-4-008	50.28	N	7.12	13.73	20.85	0.00	2.54	0.61	3.73	0.23	0.39	13.33
98-B-4-061	50.34	N	6.35	9.85	16.20	0.00	1.31	0.54	4.32	0.17	0.48	9.37

Appendix 3: Mean glucosinolate profile data for the 98-B-4 DH mapping population.

Individual	1999(a)	1999(a)	Total	Total	Total	Total	3-Butenyl	4-Pentenyl	2-Hydroxy	2-Hydroxy	3-Indolylmethyl	4-Hydroxy
	Scorings	Ratings	Alkenyl	Indolyl	Glucosinolate	Allyl			3-Butenyl	4-Pentenyl		3-indolylmethyl
			um/g	um/g	um/g oil free meal	um/g	um/g	um/g	um/g	um/g	um/g	um/g
98-B-4-197	50.48	N	1.95	4.81	6.76	0.06	0.60	0.13	1.11	0.04	0.11	4.70
98-B-4-128	50.94	N	6.43	7.77	14.19	0.02	1.76	0.69	3.86	0.10	0.35	7.42
98-B-4-118	51.18	N	6.05	11.27	17.32	0.00	1.36	0.59	3.93	0.17	0.47	10.80
98-B-4-069	51.83	N	8.42	10.25	18.67	0.02	2.26	0.54	5.40	0.20	0.95	9.30
98-B-4-126	52.83	N	3.93	5.17	9.10	0.00	1.04	0.22	2.59	0.08	0.20	4.97
98-B-4-175	52.97	N	5.62	8.44	14.06	0.01	1.38	0.38	3.56	0.28	0.25	8.19
98-B-4-089	53.14	N	11.96	9.53	21.49	0.07	4.02	0.99	6.45	0.43	0.36	9.18
98-B-4-010	53.59	N	14.31	9.78	24.09	0.00	3.29	1.26	9.10	0.66	0.48	9.30
98-B-4-132	53.77	N	17.14	11.28	28.42	0.06	5.06	0.62	11.08	0.33	0.57	10.70
98-B-4-120	54.48	N	2.68	14.17	16.85	0.00	0.99	0.38	1.25	0.06	0.76	13.41
98-B-4-088	54.97	N	33.05	9.87	42.93	0.14	5.93	1.72	23.95	1.31	0.46	9.42
98-B-4-117	55.90	N	4.73	14.88	19.60	0.00	1.36	0.49	2.77	0.11	2.90	11.98
98-B-4-109	57.58	N	4.41	10.89	15.29	0.09	1.45	0.52	2.19	0.15	0.66	10.22
98-B-4-047	57.88	N	12.00	11.91	23.91	0.04	2.92	0.39	8.19	0.46	0.62	11.29
98-B-4-112	58.33	N	9.89	10.89	20.78	0.12	3.01	1.16	5.09	0.52	0.99	9.89
98-B-4-059	58.90	N	5.22	10.85	16.07	0.00	1.25	0.55	3.25	0.16	0.84	10.02
98-B-4-108	59.34	N	2.81	11.70	14.51	0.00	0.24	0.28	1.20	1.09	0.77	10.94
98-B-4-127	60.85	N	0.69	4.97	5.66	0.00	0.28	0.04	0.38	0.00	0.22	4.75
98-B-4-111	61.11	N	8.55	10.98	19.53	0.17	3.04	0.87	4.19	0.28	0.71	10.27
98-B-4-110	61.36	N	2.65	7.00	9.65	0.15	0.83	0.28	1.30	0.08	0.20	6.81
98-B-4-092	61.93	N	12.52	14.41	26.93	0.07	4.05	1.17	7.08	0.14	1.24	13.16
98-B-4-113	63.13	S	8.47	11.86	20.33	0.09	3.60	0.97	3.66	0.15	0.90	10.96
98-B-4-067	63.61	S	0.71	4.83	5.54	0.00	0.27	0.02	0.42	0.00	0.05	4.77
98-B-4-068	64.14	S	7.52	9.30	16.82	0.04	1.86	1.03	4.15	0.44	0.33	8.98
98-B-4-101	64.20	N	3.77	11.64	15.41	0.08	1.40	0.47	1.74	0.08	1.65	9.99
98-B-4-135	66.21	S	3.49	11.36	14.85	0.00	1.30	0.20	1.92	0.06	0.55	10.81
98-B-4-103	68.18	S	6.79	11.03	17.82	0.12	2.08	1.21	3.00	0.39	1.24	9.79
98-B-4-081	68.32	S	3.15	10.57	13.72	0.00	0.94	0.17	2.02	0.02	0.25	10.32
98-B-4-090	70.16	S	13.86	10.03	23.89	0.05	2.93	1.00	9.41	0.46	0.43	9.60
98-B-4-084	70.42	S	4.53	13.40	17.94	0.08	1.33	0.19	2.87	0.07	1.11	12.29
98-B-4-072	71.20	S	7.60	10.25	17.85	0.08	1.65	1.12	4.39	0.36	0.53	9.72
98-B-4-065	71.47	S	18.59	10.96	29.55	0.11	6.10	2.31	9.31	0.76	0.54	10.42
98-B-4-080	71.73	S	11.78	11.82	23.60	0.00	2.29	0.96	7.96	0.57	1.36	10.46
98-B-4-022	74.32	S	4.77	11.39	16.15	0.00	1.95	0.67	2.10	0.05	0.52	10.87
98-B-4-074	76.18	S	7.08	12.14	19.21	0.00	2.08	0.96	3.77	0.26	0.68	11.45
98-B-4-104	78.98	S	5.97	12.73	18.70	0.09	1.82	0.84	2.94	0.27	1.44	11.29
SAL-011 (*S.alba*)	26.41	R	2.20	1.11	3.31	0.03	0.06	0.00	2.02	0.08	0.34	0.78
SAL-016 (S.alba)	28.04	R	3.59	1.52	5.11	0.07	0.09	0.00	3.43	0.00	0.43	1.08
SAL-027 (S.alba)	30.87	R	2.64	1.39	4.03	0.13	0.16	0.00	2.30	0.05	0.38	1.01
SAL-044 (S.alba)	27.04	R	2.71	1.49	4.21	0.12	0.18	0.00	2.33	0.08	0.36	1.13
Check (Average)			13.97	12.27	26.24	1.30	4.19	0.89	7.23	0.37	1.21	11.05
Correlation to 1999(a) scorings			-0.02	0.38	0.16	-0.15	0.05	0.01	-0.04	-0.13	0.04	0.42

Appendix 4: The SAS System Analysis of Variance Procedure Output

Dependent Variable: % OIL

```
Source             DF      Sum of Squares       Mean Square   F Value    Pr > F

Model              93      3139.82479389        33.76155692   31.07      0.0001

Error             230      249.95056167         1.08674157

Corrected Total       323       3389.77535556

           R-Square            C.V.          Root MSE         OIL Mean

           0.926263            2.492531      1.04246898       41.82370370


Source             DF          Anova SS        Mean Square   F Value    Pr > F

SAMPLE                93       3139.82479389       33.76155692   31.07     0.0001
```

Dependent Variable: % Protein

```
Source             DF      Sum of Squares       Mean Square   F Value    Pr > F

Model              93      1177.84614222        12.66501228   19.03      0.0001

Error             230      153.09142167         0.66561488

Corrected Total       323       1330.93756389

           R-Square            C.V.          Root MSE         PRO Mean

           0.884975            2.854525      0.81585224       28.58101852


Source             DF          Anova SS        Mean Square   F Value    Pr > F

SAMPLE                93       1177.84614222       12.66501228   19.03     0.0001
```

Dependent Variable: % Defatted Protein

```
Source              DF      Sum of Squares       Mean Square   F Value   Pr > F

Model               93      1288.81312722        13.85820567    12.24    0.0001

Error              230       260.34222833         1.13192273

Corrected Total    323      1549.15535556

          R-Square          C.V.          Root MSE        DEFPRO Mean

          0.831946          2.169950      1.06391857      49.02962963

Source              DF      Anova SS             Mean Square   F Value   Pr > F

SAMPLE              93      1288.81312722        13.85820567    12.24    0.0001
```

Dependent Variable: % Dry Matter

```
Source              DF      Sum of Squares       Mean Square   F Value   Pr > F

Model               93      63.02603056          0.67769925     68.65    0.0001

Error              230       2.27045834          0.00987156

Corrected Total    323      65.29648890

          R-Square          C.V.          Root MSE        DM Mean

          0.965228          0.105126      0.09935571      94.51148148

Source              DF      Anova SS             Mean Square   F Value   Pr > F

SAMPLE              93      63.02603056          0.67769925     68.65    0.0001
```

Dependent Variable: Alkenyl (Aliphatics)

```
Source            DF     Sum of Squares       Mean Square   F Value    Pr > F

Model             91     42046.26295958       462.04684571   122.26    0.0001

Error             90     340.14247778         3.77936086

Corrected Total      181     42386.40543736

         R-Square           C.V.          Root MSE        ALK Mean

         0.991975           19.36800      1.94405783      10.03747253

Source            DF         Anova SS        Mean Square   F Value    Pr > F

SAMPLE               91     42046.26295958       462.04684571    122.26    0.0001
```

Dependent Variable: Indolyls

```
Source            DF     Sum of Squares       Mean Square   F Value    Pr > F

Model             91     1481.15670531        16.27644731     8.70    0.0001

Error           90      168.29351667        1.86992796

Corrected Total      181     1649.45022198

         R-Square           C.V.          Root MSE        INDOS Mean

         0.897970           13.26761      1.36745309      10.30670330

Source            DF         Anova SS        Mean Square   F Value    Pr > F

SAMPLE               91     1481.15670531        16.27644731     8.70    0.0001
```

Dependent Variable: Total Glucosinolate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	43652.10967937	479.69351296	95.35	0.0001
Error	90	452.76817778	5.03075753		
Corrected Total	181	44104.87785714			

R-Square	C.V.	Root MSE	GLUC Mean
0.989734	11.02489	2.24293503	20.34428571

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SAMPLE	91	43652.10967937	479.69351296	95.35	0.0001

Dependent Variable: Allyls

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	27.72280299	0.30464619	105.61	0.0001
Error	90	0.25962778	0.00288475		
Corrected Total	181	27.98243077			

R-Square	C.V.	Root MSE	ALLYL Mean
0.990722	27.16843	0.05370990	0.19769231

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SAMPLE	91	27.72280299	0.30464619	105.61	0.0001

Dependent Variable: 3-Butenyl

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	22548.72658669	247.78820425	1164.48	0.0001
Error	90	19.15101111	0.21278901		
Corrected Total	181	22567.87759780			

```
          R-Square          C.V.          Root MSE          BUT3 Mean

          0.999151          13.67611      0.46129059        3.37296703

Source              DF      Anova SS           Mean Square    F Value    Pr > F

SAMPLE              91      22548.72658669     247.78820425   1164.48    0.0001
```

Dependent Variable: 4-Pentenyl

```
Source              DF      Sum of Squares     Mean Square    F Value    Pr > F

Model               91      2203.34682753      24.21260250    159.24     0.0001

Error               90      13.68419444        0.15204660

Corrected Total     181     2217.03102198

          R-Square          C.V.          Root MSE          PENT4 Mean

          0.993828          35.23361      0.38993154        1.10670330

Source              DF      Anova SS           Mean Square    F Value    Pr > F

SAMPLE              91      2203.34682753      24.21260250    159.24     0.0001
```

Dependent Variable: 2-Hydroxy 3-Butenyl

```
Source              DF      Sum of Squares     Mean Square    F Value    Pr > F

Model               91      2214.95162991      24.34012780    17.10      0.0001

Error               90      128.11827778       1.42353642

Corrected Total     181     2343.06990769

          R-Square          C.V.          Root MSE          OHBUT Mean

          0.945320          23.83308      1.19312045        5.00615385

Source              DF      Anova SS           Mean Square    F Value    Pr > F

SAMPLE              91      2214.95162991      24.34012780    17.10      0.0001
```

Dependent Variable: 2-Hydroxy 4-Pentenyl

```
Source              DF     Sum of Squares      Mean Square   F Value   Pr > F

Model               91      19.49472753        0.21422778      3.08    0.0001

Error               90      6.25844444         0.06953827

Corrected Total    181       25.75317198

          R-Square          C.V.          Root MSE        OHPEN Mean

          0.756984          74.48952      0.26370110       0.35401099


Source              DF        Anova SS         Mean Square   F Value   Pr > F

SAMPLE              91      19.49472753        0.21422778      3.08    0.0001
```

Dependent Variable: 3-indolylmethyl

```
Source              DF     Sum of Squares      Mean Square   F Value   Pr > F

Model               91      59.10347479        0.64948873     12.13    0.0001

Error               90      4.81964444         0.05355160

Corrected Total    181       63.92311923

          R-Square          C.V.          Root MSE        INDO3 Mean

          0.924602          29.16489      0.23141220       0.79346154


Source              DF        Anova SS         Mean Square   F Value   Pr > F

SAMPLE              91      59.10347479        0.64948873     12.13    0.0001
```

Dependent Variable: 4-Hydroxy 3-indolylmethyl

```
Source              DF     Sum of Squares      Mean Square   F Value   Pr > F

Model               91     1136.91000336       12.49351652     7.55    0.0001

Error               90     149.00799444        1.65564438

Corrected Total    181      1285.91799780

          R-Square          C.V.          Root MSE        OHIN4 Mean
```

```
               0.884123           13.52594         1.28671846        9.51296703

Source             DF          Anova SS          Mean Square   F Value    Pr > F

SAMPLE               91      1136.91000336          12.49351652      7.55     0.0001

                         Dependent Variable: C160 Palmitic

Source             DF        Sum of Squares        Mean Square   F Value    Pr > F

Model               88          65.60316719         0.74549054    16.96    0.0001

Error              231         10.15482500         0.04396028

Corrected Total      319          75.75799219

               R-Square              C.V.          Root MSE        C160 Mean

               0.865957            5.158060        0.20966707       4.06484375

Source             DF          Anova SS          Mean Square   F Value    Pr > F

SAMPLE               88          65.60316719          0.74549054    16.96     0.0001

                         Dependent Variable: C161 Palmitoleic

Source             DF        Sum of Squares        Mean Square   F Value    Pr > F

Model               88           0.67943000         0.00772080    12.51    0.0001

Error              231           0.14252500         0.00061699

Corrected Total      319           0.82195500

               R-Square              C.V.          Root MSE        C161 Mean

               0.826602            9.476132        0.02483931       0.26212500

Source             DF          Anova SS          Mean Square   F Value    Pr > F

SAMPLE               88           0.67943000          0.00772080    12.51     0.0001
```

Dependent Variable: C180 Stearic

```
Source             DF     Sum of Squares       Mean Square   F Value    Pr > F

Model              88       9.54827219         0.10850309    22.11     0.0001

Error             231       1.13382500         0.00490833

Corrected Total   319        10.68209719

         R-Square           C.V.         Root MSE        C180 Mean

         0.893857         4.898944      0.07005950       1.43009375

Source             DF          Anova SS       Mean Square   F Value    Pr > F

SAMPLE                88     9.54827219        0.10850309    22.11     0.0001
```

Dependent Variable: C181 Oleic

```
Source             DF     Sum of Squares       Mean Square   F Value    Pr > F

Model              88    20835.05039500      236.76193631   179.05    0.0001

Error             231     305.44920000         1.32229091

Corrected Total   319      21140.49959500

         R-Square           C.V.         Root MSE        C181 Mean

         0.985551         2.134308      1.14990909      53.87737500

Source             DF          Anova SS       Mean Square   F Value    Pr > F

SAMPLE                88   20835.05039500      236.76193631   179.05    0.0001
```

Dependent Variable: C182 Linoleic

```
Source             DF     Sum of Squares       Mean Square   F Value    Pr > F

Model              88     3228.93956719       36.69249508    61.71    0.0001

Error             231     137.35092500        0.59459275

Corrected Total   319       3366.29049219

         R-Square           C.V.         Root MSE        C182 Mean
```

```
          0.959198          3.454766        0.77109840       22.31984375

Source          DF          Anova SS        Mean Square  F Value   Pr > F

SAMPLE              88      3228.93956719       36.69249508   61.71    0.0001
```

Dependent Variable: C183 Linolenic

```
Source          DF        Sum of Squares        Mean Square  F Value    Pr > F

Model           88         817.19635500         9.28632222   14.79     0.0001

Error          231        145.00140000         0.62771169

Corrected Total     319        962.19775500

          R-Square          C.V.          Root MSE        C183 Mean

          0.849302          6.538536      0.79228258      12.11712500

Source          DF          Anova SS        Mean Square  F Value   Pr > F

SAMPLE              88      817.19635500        9.28632222   14.79    0.0001
```

Dependent Variable: C200 Arachidic

```
Source          DF        Sum of Squares        Mean Square  F Value    Pr > F

Model           88          2.98785469        0.03395289   10.56    0.0001

Error          231          0.74245000        0.00321407

Corrected Total     319          3.73030469

          R-Square          C.V.          Root MSE        C200 Mean

          0.800968          9.787798      0.05669276       0.57921875

Source          DF          Anova SS        Mean Square  F Value   Pr > F

SAMPLE              88       2.98785469         0.03395289   10.56    0.0001
```

Dependent Variable: C201 Gadoleic

```
Source          DF        Sum of Squares        Mean Square  F Value    Pr > F

Model           88        3595.92061375       40.86273425  1333.35    0.0001
```

```
Error              231          7.07937500          0.03064665

Corrected Total        319          3602.99998875

          R-Square            C.V.          Root MSE        C201 Mean

          0.998035            7.627343      0.17506183        2.29518750

Source             DF          Anova SS          Mean Square  F Value    Pr > F

SAMPLE                88          3595.92061375          40.86273425  1333.35    0.0001
```

Dependent Variable: C202 Eicosadienoic

```
Source             DF          Sum of Squares          Mean Square  F Value    Pr > F

Model              88          16.20020500          0.18409324    24.77    0.0001

Error              231         1.71695000          0.00743268

Corrected Total        319          17.91715500

          R-Square            C.V.          Root MSE        C202 Mean

          0.904173            44.12694      0.08621302        0.19537500

Source             DF          Anova SS          Mean Square  F Value    Pr > F

SAMPLE                88          16.20020500          0.18409324    24.77    0.0001
```

Dependent Variable: C220 Behenic

```
Source             DF          Sum of Squares          Mean Square  F Value    Pr > F

Model              88          5.11353719          0.05810838    37.31    0.0001

Error              231         0.35972500          0.00155725

Corrected Total        319          5.47326219

          R-Square            C.V.          Root MSE        C220 Mean

          0.934276            10.23243      0.03946202        0.38565625

Source             DF          Anova SS          Mean Square  F Value    Pr > F

SAMPLE                88          5.11353719          0.05810838    37.31    0.0001
```

Dependent Variable: C221 Erucic

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	88	15119.14711875	171.80848999	22522.35	0.0001
Error	231	1.76215000	0.00762835		
Corrected Total	319	15120.90926875			

R-Square	C.V.	Root MSE	C221 Mean
0.999883	4.542328	0.08734045	1.92281250

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SAMPLE	88	15119.14711875	171.80848999	22522.35	0.0001

Dependent Variable: C240 Lignoceric

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	88	0.33436375	0.00379959	13.39	0.0001
Error	231	0.06552500	0.00028366		
Corrected Total	319	0.39988875			

R-Square	C.V.	Root MSE	C240 Mean
0.836142	9.717793	0.01684215	0.17331250

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SAMPLE	88	0.33436375	0.00379959	13.39	0.0001

Dependent Variable: C241 Nervonic

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	88	37.21043000	0.42284580	898.18	0.0001
Error	231	0.10875000	0.00047078		
Corrected Total	319	37.31918000			

R-Square	C.V.	Root MSE	C241 Mean

```
            0.997086          6.671006          0.02169745        0.32525000

Source            DF          Anova SS          Mean Square  F Value   Pr >F

SAMPLE                88      37.21043000          0.42284580  898.18   0.0001
```

Dependent Variable: Total Saturates

```
Source            DF          Sum of Squares      Mean Square  F Value   Pr > F

Model             88          115.17663469        1.30882539   18.01    0.0001

Error            231          16.78517500       0.07266310

Corrected Total       319         131.96180969

            R-Square          C.V.          Root MSE        TOTSAT Mean

            0.872803          4.173113      0.26956093        6.45946875

Source            DF          Anova SS          Mean Square  F Value   Pr > F

SAMPLE                88      115.17663469         1.30882539   18.01    0.0001
```

Appendix 5: Mean whole seed NIR quality analysis data for the 98-B-4 DH mapping population

Individual	1999(a)	1999(a)	Moisture	Oil	Protein	Defatted	Total	Dry Matter
	Scorings	Ratings				Protein	Glucosinolate	
			%	%	%	%		%
LK-001	49.09	N	6.15	38.12	30.02	48.09	18.88	93.83
94-99	50.95	N	6.20	37.33	31.82	50.26	11.86	93.78
98-B-4-242	24.87	R	5.59	44.82	26.78	48.15	9.18	94.38
98-B-4-243	26.50	R	5.81	43.20	27.49	47.97	16.56	94.14
98-B-4-189	28.80	R	5.93	41.74	27.70	46.59	10.46	94.06
98-B-4-176	31.34	R	6.02	39.96	30.05	50.06	8.91	94.00
98-B-4-107	33.01	R	5.20	40.99	28.55	48.02	14.89	94.82
98-B-4-246	33.84	R	5.37	44.02	27.45	48.27	9.29	94.63
98-B-4-195	35.86	R	5.90	41.38	30.01	51.15	7.54	94.09
98-B-4-187	36.39	N	5.25	45.93	26.04	47.57	5.54	94.71
98-B-4-178	36.58	R	5.67	40.65	30.35	50.50	9.84	94.37
98-B-4-190	36.91	N	5.93	41.94	27.91	48.20	15.80	94.04
98-B-4-247	37.10	R	5.71	42.50	28.41	48.96	7.46	94.29
98-B-4-181	39.18	N	5.65	40.91	28.90	48.51	7.30	94.36
98-B-4-172	39.95	R	5.75	43.05	27.03	46.90	7.27	94.24
98-B-4-064	40.41	N	5.33	43.05	26.31	46.21	14.78	94.69
98-B-4-194	40.58	N	5.91	41.66	29.30	50.26	8.87	94.07
98-B-4-180	41.13	N	5.71	39.88	28.74	47.45	13.75	94.28
98-B-4-174	41.55	N	5.69	41.60	30.40	51.86	21.71	94.28
98-B-4-241	42.20	N	5.77	39.40	30.07	50.00	13.92	94.22
98-B-4-116	42.58	N	5.58	40.74	29.66	50.41	12.84	94.41
98-B-4-157	43.38	N	5.28	47.76	25.42	47.90	5.92	94.72
98-B-4-245	43.62	N	5.29	42.45	28.82	50.42	5.22	94.71
98-B-4-179	43.72	N	5.86	40.30	28.97	48.28	10.21	94.10
98-B-4-133	44.29	N	6.12	40.26	29.68	50.05	16.78	93.88
98-B-4-182	44.37	N	5.74	40.12	31.37	53.08	7.30	94.26
98-B-4-003	44.48	N	5.21	44.99	26.65	48.57	11.57	94.77
98-B-4-017	45.21	N	4.89	47.89	24.16	46.81	9.67	95.09
98-B-4-192	45.29	N	5.55	43.78	26.95	47.69	9.98	94.45
98-B-4-184	45.29	N	5.00	47.40	24.91	46.68	11.67	94.99
98-B-4-014	46.13	N	5.06	45.28	26.38	48.69	9.89	94.94
98-B-4-004	46.13	N	4.81	46.12	23.54	43.23	9.01	95.17
98-B-4-244	46.48	N	5.73	42.70	29.08	50.52	6.72	94.25

Appendix 5: Mean whole seed NIR quality analysis data for the 98-B-4 DH mapping population

Individual	1999(a)	1999(a)	Moisture	Oil	Protein	Defatted	Total	Dry Matter
	Scorings	Ratings				Protein	Glucosinolate	
			%	%	%	%		%
98-B-4-099	48.01	N	5.00	46.19	26.63	49.97	13.53	94.97
98-B-4-070	48.17	N	5.31	40.00	29.17	48.72	13.05	94.72
98-B-4-009	48.62	N	5.30	41.12	28.09	47.77	16.44	94.69
98-B-4-173	48.86	N	6.04	39.89	28.65	47.65	15.94	93.95
98-B-4-191	49.48	N	5.99	41.03	30.29	51.89	8.76	94.00
98-B-4-185	49.52	N	5.59	43.63	28.03	49.00	6.69	94.39
98-B-4-063	50.00	N	5.16	39.98	27.12	45.11	20.11	94.84
98-B-4-073	50.00	N	5.10	43.60	28.56	51.15	11.02	94.87
98-B-4-008	50.28	N	5.03	44.33	26.93	48.68	10.42	94.96
98-B-4-061	50.34	N	5.26	42.27	28.16	48.88	11.11	94.72
98-B-4-197	50.48	N	5.79	41.00	30.23	51.20	5.59	94.21
98-B-4-128	50.94	N	5.83	41.49	28.15	47.44	7.22	94.16
98-B-4-118	51.18	N	5.61	41.43	27.81	47.13	9.88	94.42
98-B-4-069	51.83	N	5.25	37.04	30.37	47.45	15.00	94.78
98-B-4-126	52.83	N	5.74	41.63	28.56	48.95	10.76	94.23
98-B-4-175	52.97	N	5.62	43.08	28.40	48.97	7.17	94.37
98-B-4-089	53.14	N	5.17	43.11	29.53	51.78	12.19	94.86
98-B-4-010	53.59	N	4.99	41.95	28.28	47.76	13.55	95.03
98-B-4-132	53.77	N	5.91	39.51	30.29	49.93	13.99	94.06
98-B-4-120	54.48	N	5.73	42.48	28.84	50.21	10.68	94.29
98-B-4-088	54.97	N	5.39	37.83	31.17	50.25	23.31	94.57
98-B-4-117	55.90	N	5.47	41.16	26.75	44.88	14.15	94.53
98-B-4-109	57.58	N	5.62	40.40	30.83	52.36	10.34	94.35
98-B-4-047	57.88	N	5.03	43.81	26.84	47.29	18.00	94.91
98-B-4-112	58.33	N	4.97	40.91	30.30	51.87	12.90	95.03
98-B-4-059	58.90	N	4.98	42.69	28.70	50.60	11.13	95.00
98-B-4-108	59.34	N	4.50	46.58	25.84	48.52	7.87	95.49
98-B-4-127	60.85	N	5.78	42.00	28.89	49.97	9.08	94.17
98-B-4-111	61.11	N	5.59	41.18	29.78	51.31	13.76	94.41
98-B-4-110	61.36	N	4.99	43.51	28.10	49.55	7.43	94.99
98-B-4-092	61.93	N	5.37	41.78	29.91	52.54	15.82	94.62
98-B-4-113	63.13	S	5.24	42.08	28.74	49.52	11.95	94.75
98-B-4-067	63.61	S	4.92	43.83	26.90	47.85	7.39	95.07
98-B-4-068	64.14	S	5.25	40.73	28.44	47.97	11.31	94.81
98-B-4-101	64.20	N	5.20	40.66	29.31	50.07	12.42	94.82

Appendix 5: Mean whole seed NIR quality analysis data for the 98-B-4 DH mapping population

Individual	1999(a) Scorings	1999(a) Ratings	Moisture %	Oil %	Protein %	Defatted Protein %	Total Glucosinolate	Dry Matter %
98-B-4-135	66.21	S	5.58	43.40	28.58	50.58	6.15	94.41
98-B-4-103	68.18	S	5.48	42.48	30.03	52.61	8.94	94.52
98-B-4-081	68.32	S	4.96	42.78	28.36	49.49	10.09	95.02
98-B-4-090	70.16	S	5.01	41.33	28.58	48.67	14.47	94.98
98-B-4-084	70.42	S	5.08	44.31	27.13	48.61	9.95	94.90
98-B-4-072	71.20	S	4.71	45.41	25.63	46.47	9.36	95.27
98-B-4-065	71.47	S	5.20	43.55	28.72	50.99	14.82	94.76
98-B-4-080	71.73	S	5.03	42.80	28.89	50.50	13.23	94.98
98-B-4-022	74.32	S	5.31	39.44	31.00	51.27	12.23	94.70
98-B-4-074	76.18	S	5.23	41.65	29.58	51.36	11.45	94.78
98-B-4-104	78.98	S	5.27	42.38	27.71	47.99	11.87	94.75
SAL-011 (*S.alba*)	26.41	R	7.09	25.53	37.63	52.01	39.86	92.81
SAL-016 (*S.alba*)	28.04	R	6.48	30.49	31.28	42.85	40.28	93.43
SAL-027 (*S.alba*)	30.87	R	6.50	29.86	33.39	47.34	38.55	93.44
SAL-044 (*S.alba*)	27.04	R	6.67	28.83	34.92	49.78	36.55	93.25
Correlation to 1999 (a) scorings			-0.58	0.35	-0.20	0.23	-0.32	0.58

Appendix 6: Mean whole seed fatty acid profile for the 98-B-4 DH mapping population

Individual	1999(a) Scorings	C16:0 Palmitic	C16:1 Palmitoleic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic	C20:0 Arachidic	C20:1 Gadoleic	C20:2 Eicosadienoic	C22:0 Behenic	C22:1 Erucic	C24:0 Lignoceric	C24:1 Nervonic	Total Saturates
		%	%	%	%	%	%	%	%	%	%	%	%	%	%
LK-001	49.09	4.29	0.32	1.84	58.24	20.64	7.84	0.72	2.61	0.14	0.51	2.22	0.24	0.32	7.37
94-99	50.95	4.46	0.28	1.52	59.39	21.77	9.53	0.57	1.27	0.08	0.30	0.34	0.20	0.23	6.85
98-B-4-242	24.87	4.05	0.22	1.45	56.16	21.88	13.73	0.52	1.28	0.10	0.32	0.04	0.17	0.23	6.34
98-B-4-243	26.50	4.37	0.26	1.64	58.61	19.68	12.79	0.55	1.18	0.08	0.32	0.03	0.18	0.27	6.88
98-B-4-189	28.80	4.09	0.33	1.36	57.98	19.80	13.52	0.55	1.40	0.09	0.35	0.05	0.21	0.23	6.35
98-B-4-176	31.34	4.04	0.29	1.24	54.44	22.13	14.69	0.55	1.56	0.12	0.38	0.04	0.22	0.27	6.21
98-B-4-107	33.01	4.37	0.32	1.41	56.20	21.46	13.35	0.57	1.39	0.10	0.38	0.03	0.16	0.20	6.73
98-B-4-246	33.84	4.00	0.25	1.56	60.48	19.94	11.03	0.56	1.28	0.09	0.35	0.05	0.16	0.21	6.47
98-B-4-195	35.86	4.60	0.30	1.44	55.02	23.03	12.52	0.54	1.43	0.12	0.37	0.06	0.21	0.29	6.98
98-B-4-187	36.39	4.15	0.25	1.42	58.47	21.15	12.06	0.51	1.22	0.07	0.27	0.03	0.13	0.22	6.36
98-B-4-178	36.58	4.28	0.36	1.55	55.94	22.52	12.41	0.60	1.30	0.10	0.39	0.02	0.22	0.25	6.82
98-B-4-190	36.91	3.80	0.23	1.58	56.54	24.04	10.91	0.58	1.35	0.09	0.35	0.04	0.18	0.26	6.29
98-B-4-247	37.10	3.79	0.22	1.47	58.44	19.82	13.26	0.55	1.48	0.09	0.35	0.04	0.18	0.26	6.16
98-B-4-181	39.18	4.15	0.34	1.32	54.42	24.17	12.52	0.56	1.48	0.14	0.37	0.04	0.20	0.26	6.40
98-B-4-172	39.95	4.16	0.25	1.49	56.67	22.98	11.31	0.57	1.52	0.13	0.40	0.07	0.17	0.24	6.62
98-B-4-064	40.41	4.17	0.24	1.60	57.50	22.40	11.12	0.63	1.43	0.10	0.37	0.03	0.15	0.19	6.77
98-B-4-194	40.58	4.10	0.24	1.26	52.37	27.01	11.98	0.49	1.45	0.14	0.38	0.08	0.15	0.29	6.24
98-B-4-160	41.13	5.08	0.28	1.50	54.86	26.16	9.21	0.64	1.17	0.14	0.45	0.04	0.17	0.22	7.68
98-B-4-174	41.55	3.68	0.29	0.99	20.37	18.40	12.00	0.58	14.03	0.89	0.31	27.09	0.15	1.16	5.56
98-B-4-241	42.20	3.79	0.23	1.43	54.50	24.54	12.48	0.51	1.40	0.12	0.35	0.06	0.24	0.30	6.08
98-B-4-116	42.58	4.18	0.27	1.60	54.58	23.22	13.27	0.57	1.26	0.10	0.36	0.04	0.20	0.30	6.71
98-B-4-157	43.38	3.81	0.24	1.68	61.85	19.77	10.09	0.53	1.16	0.10	0.29	0.06	0.16	0.21	6.32
98-B-4-245	43.62	4.03	0.28	1.36	54.23	26.11	10.79	0.55	1.52	0.15	0.39	0.07	0.19	0.29	6.32
98-B-4-179	43.72	4.28	0.24	1.31	52.70	27.67	10.68	0.54	1.52	0.15	0.36	0.03	0.19	0.26	6.50
98-B-4-133	44.29	3.72	0.22	1.34	57.39	21.56	12.83	0.48	1.48	0.13	0.31	0.07	0.18	0.28	5.84
98-B-4-182	44.37	4.57	0.30	1.19	49.13	27.48	14.53	0.56	1.13	0.15	0.43	0.08	0.15	0.28	6.74
98-B-4-003	44.48	3.88	0.25	1.24	57.39	20.87	13.45	0.64	1.48	0.10	0.34	0.03	0.11	0.18	6.09
98-B-4-017	45.21	3.71	0.23	1.52	64.43	17.40	10.17	0.55	1.28	0.06	0.28	0.03	0.14	0.16	6.06
98-B-4-192	45.29	4.12	0.22	1.54	57.73	23.37	9.60	0.60	1.62	0.13	0.43	0.06	0.18	0.24	6.70
98-B-4-184	45.29	2.88	0.20	1.06	23.17	14.24	9.88	0.64	16.29	0.66	0.31	29.45	0.13	1.02	4.90
98-B-4-004	46.13	4.01	0.25	1.35	59.95	21.41	10.33	0.52	1.36	0.10	0.32	0.02	0.13	0.20	6.20
98-B-4-014	46.13	3.79	0.23	1.43	62.64	18.57	10.71	0.53	1.33	0.07	0.30	0.02	0.15	0.18	6.05
98-B-4-244	46.48	4.05	0.29	1.27	54.79	23.03	13.76	0.46	1.33	0.13	0.31	0.06	0.20	0.27	6.09
98-B-4-099	48.01	3.79	0.21	1.40	55.16	24.13	12.55	0.52	1.36	0.10	0.31	0.02	0.16	0.24	6.03
98-B-4-070	48.17	4.39	0.30	1.43	50.89	26.29	12.87	0.64	1.52	0.40	0.51	0.24	0.18	0.28	6.97
98-B-4-009	48.62	4.11	0.22	1.55	54.34	24.38	12.32	0.62	1.54	0.13	0.38	0.02	0.15	0.22	6.65
98-B-4-173	48.86	4.16	0.29	1.48	55.81	22.35	12.77	0.59	1.49	0.11	0.37	0.04	0.22	0.25	6.61
98-B-4-191	49.48	4.18	0.27	1.46	52.25	24.47	14.31	0.55	1.40	0.11	0.39	0.13	0.14	0.29	6.58
98-B-4-185	49.52	4.40	0.26	1.29	54.86	23.83	12.48	0.54	1.39	0.11	0.36	0.09	0.13	0.20	6.60
98-B-4-073	50.00	4.02	0.24	1.44	59.05	20.37	11.96	0.53	1.28	0.16	0.40	0.13	0.14	0.22	6.40
98-B-4-063	50.00	4.42	0.32	1.89	59.20	16.99	14.20	0.70	1.38	0.07	0.39	0.07	0.15	0.18	7.40
98-B-4-008	50.28	3.58	0.19	1.25	56.08	23.63	12.37	0.52	1.53	0.12	0.35	0.02	0.11	0.20	5.71
98-B-4-061	50.34	4.38	0.28	1.73	58.06	18.69	14.10	0.61	1.22	0.07	0.32	0.06	0.19	0.24	7.04
98-B-4-197	50.48	4.41	0.35	1.12	52.14	25.95	13.07	0.46	1.46	0.13	0.35	0.05	0.17	0.28	6.33
98-B-4-128	50.94	4.51	0.37	1.40	51.89	25.19	13.71	0.50	1.40	0.12	0.32	0.04	0.21	0.29	6.73
98-B-4-118	51.18	4.63	0.35	1.41	53.26	24.91	12.23	0.55	1.42	0.16	0.43	0.07	0.20	0.31	7.02
98-B-4-069	51.83	5.27	0.35	1.48	47.93	28.54	12.09	0.73	1.51	0.67	0.62	0.25	0.18	0.28	8.11
98-B-4-126	52.83	4.01	0.23	1.44	56.31	22.52	12.52	0.53	1.38	0.12	0.36	0.06	0.20	0.26	6.34
98-B-4-175	52.97	4.67	0.35	1.33	55.87	23.44	11.33	0.55	1.46	0.12	0.37	0.07	0.16	0.22	6.92
98-B-4-089	53.14	3.96	0.28	1.34	53.85	25.60	12.28	0.49	1.29	0.12	0.30	0.03	0.17	0.26	6.09
98-B-4-010	53.59	4.34	0.29	1.33	55.48	23.47	12.30	0.56	1.31	0.11	0.37	0.01	0.14	0.25	6.59
98-B-4-132	53.77	3.74	0.19	1.52	57.38	23.86	10.28	0.54	1.43	0.15	0.35	0.06	0.17	0.27	6.15
98-B-4-120	54.48	4.28	0.30	1.45	55.49	23.80	11.84	0.51	1.35	0.13	0.33	0.04	0.17	0.25	6.57

Appendix 6: Mean whole seed fatty acid profile for the 98-B-4 DH mapping population

Individual	1999(a)	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	Total
	Scorings	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Arachidic	Gadoleic	Eicosadienoic	Behenic	Erucic	Lignoceric	Nervonic	Saturates
98-B-4-088	54.97	4.37	0.25	1.32	55.80	21.39	13.91	0.48	1.34	0.17	0.34	0.07	0.22	0.30	6.51
98-B-4-117	55.90	4.57	0.30	1.81	52.20	25.77	12.28	0.61	1.24	0.12	0.41	0.05	0.26	0.32	7.40
98-B-4-109	57.58	4.14	0.28	1.53	55.64	21.70	13.79	0.59	1.36	0.10	0.34	0.03	0.20	0.26	6.60
98-B-4-047	57.86	2.81	0.16	1.09	23.02	16.97	9.84	0.64	15.05	0.79	0.34	28.03	0.16	1.03	4.60
98-B-4-112	58.33	3.65	0.18	1.48	53.42	25.87	12.32	0.57	1.54	0.14	0.33	0.04	0.17	0.27	6.02
98-B-4-059	58.90	3.91	0.22	1.54	57.61	21.78	11.94	0.59	1.46	0.10	0.34	0.05	0.18	0.24	6.38
98-B-4-108	59.34	4.01	0.25	1.38	57.84	22.89	10.98	0.55	1.35	0.08	0.33	0.04	0.11	0.16	6.26
98-B-4-127	60.85	3.58	0.22	1.38	56.06	25.08	10.49	0.51	1.55	0.15	0.38	0.09	0.18	0.25	5.85
98-B-4-111	61.11	4.07	0.28	1.50	60.07	18.47	12.69	0.58	1.40	0.07	0.32	0.08	0.18	0.21	6.47
98-B-4-110	61.36	4.13	0.29	1.32	54.50	24.76	12.01	0.55	1.48	0.13	0.35	0.03	0.18	0.23	6.36
98-B-4-092	61.93	3.87	0.24	1.57	58.60	21.60	11.36	0.57	1.32	0.10	0.33	0.02	0.16	0.22	6.33
98-B-4-113	63.13	3.65	0.25	1.25	53.43	23.66	14.72	0.48	1.43	0.13	0.32	0.06	0.14	0.22	5.89
98-B-4-067	63.61	3.71	0.22	1.42	57.00	23.02	9.41	0.87	1.81	0.83	0.75	0.61	0.12	0.18	6.74
98-B-4-068	64.14	4.51	0.32	1.69	52.45	23.50	12.31	0.94	1.60	0.92	0.74	0.53	0.19	0.28	7.88
98-B-4-101	64.20	4.26	0.24	1.64	55.27	22.82	12.71	0.66	1.44	0.09	0.40	0.02	0.16	0.25	6.96
98-B-4-135	66.21	3.37	0.23	1.88	56.93	22.65	11.74	0.60	1.45	0.14	0.36	0.07	0.23	0.27	6.21
98-B-4-103	68.18	4.36	0.25	1.39	53.70	22.81	14.79	0.55	1.29	0.09	0.32	0.02	0.14	0.25	6.61
98-B-4-061	68.32	3.38	0.21	1.25	54.83	24.09	12.97	0.49	1.64	0.18	0.40	0.12	0.16	0.25	5.52
98-B-4-090	70.16	4.32	0.27	1.36	54.10	23.67	13.36	0.54	1.41	0.11	0.33	0.01	0.20	0.27	6.55
98-B-4-084	70.42	3.29	0.22	1.42	53.90	25.66	12.37	0.54	1.45	0.18	0.40	0.10	0.16	0.27	5.65
98-B-4-072	71.20	4.19	0.24	1.47	57.38	22.74	10.49	0.60	1.48	0.30	0.49	0.22	0.14	0.21	6.75
98-B-4-065	71.47	3.73	0.27	1.36	55.86	18.88	13.41	1.07	1.75	1.35	1.24	0.70	0.13	0.19	7.40
98-B-4-060	71.73	3.89	0.22	1.46	56.09	22.50	13.00	0.49	1.21	0.16	0.38	0.07	0.17	0.31	6.22
98-B-4-022	74.32	4.41	0.27	1.44	52.08	24.44	14.14	0.62	1.57	0.12	0.40	0.04	0.17	0.27	6.87
98-B-4-074	76.18	4.74	0.29	1.46	54.86	23.18	12.21	0.54	1.38	0.22	0.37	0.24	0.18	0.28	7.10
98-B-4-104	78.98	4.53	0.32	1.54	51.28	24.93	14.16	0.66	1.47	0.13	0.45	0.03	0.18	0.26	7.18
SAL-011 (*S.alba*)	26.41	2.12	0.20	0.73	25.33	9.96	11.68	0.52	8.04	0.28	0.54	37.23	0.28	3.06	3.90
SAL-016 (*S.alba*)	28.04	3.77	0.24	1.24	37.99	12.93	13.96	0.56	10.86	0.27	0.27	15.89	0.13	1.77	5.84
SAL-027 (*S.alba*)	30.67	2.53	0.19	1.04	29.41	10.44	12.03	0.62	9.96	0.32	0.47	30.36	0.22	2.34	4.66
Correlation to 1999 (a) scorings		0.12	-0.02	0.20	0.15	0.33	0.06	0.24	-0.19	0.20	0.25	-0.26	-0.17	-0.32	0.23

Appendix 7: Solutions and Buffers

KIRBY MIX (400ml)

p-Aminosalicyclic acid (Flourochem)	28.9g
Sodium Tri-isopropylnapthalene Sulphonate (Eastman Kodak)	4.0g
2M Tris-Hcl (pH 8.0)	10.0ml
Buffer saturated Phenol (pH 8.0)	24.0ml
(0.25 g hydroxyquinoline, 500g phenol (saturated with 2M Tris-Hcl)	
Deionised water	360.0ml

PHENOL/CHLOROFORM MIX (100ml)

Buffer saturated phenol	50.0ml
Chloroform	48.0ml
Isoamyl alcohol	2.0ml

10X TE (1L)

Tris	12.1g
EDTA	3.7g
pH 8. 0	

10X TEN (1L)

2M Tris	50.0ml
O.SM EDTA	20.0ml
3M NaCl	166.7ml

50X TAE (1L)

Tris	242.0g
Glacial acetic acid	57.1ml
0.5M EDTA (pH 8.0)	100.0ml

0.8% AGAROSE GEL (400m1)

Deionised water	392.0ml
lx TAE	8.0ml
Agarose (Sigma)	3.2g

10X LOADING BUFFER (40ml)

Ficoll 400 (Sigma)	8.0g
0.5M EDTA	6.0ml
Bromophenol blue	40.0mg

10X H BUFFER (30ml)

2M Tris Hcl (500mM)	7.5ml
lM $MgCl_2$ (100mM)	3.0ml
3M NaCl (1M)	10.0ml
0.1M DTE (10mM)	3.0ml
100x spermadine (40mM)	3.5ml
Water	3.5ml

2M TRIS HCL (1L)

Tris base	242.3g
HCl (10M)	92.0ml
pH 8.0	

DEPURINATION SOLUTION (1L)

Conc Hcl (approximately 10M)	25.0ml

ALKALI TRANSFER SOLUTION (1L)

NaOH (0.4M)	16.0g

20X SSC (1L)

NaCl (3M)	175.3g
Trisodium citrate (0.3M)	88.2g

PREHYBRIDISATION SOLUTION (30ml)

Dextran sulphate (Sigma D-6001)	0.3g
Deionised water	17.55ml
20x Sets	6.0ml
50x Denhardts	6.0ml
10% SDS (w/v in deionised water)	0.3ml
Herring Testes DNA (10mg/ml) (Sigma D-6898)	0.15ml

HYBRIDISATION SOLUTION (4.75mL)

Dextran sulphate (Sigma D-6001)	0.5g
Deionised water	2.7.ml
20x Sets	1.0ml
50x Denhardts	1.0ml
10% SDS (w/v in deionised water)	50.0μl

50X DENHARDTS (250 mL)

Ficoll (400)	2.5g
Polyvinlypyrrolidine (360)	2.5g
BSA (Fraction V)	2.5g
Deionised water	250.0ml

20X SETS (1L)

NaCl (3M)	175.3g
0.5M EDTA (pH 8.0)	40.0ml
2M Tris-Hcl (pH 8.0)	300.0ml
Tetra-sodium pyrophosphate (11mM)	5.0g

dCTP OLIGO-LABELLING BUFFER (500μl)

0.5M Tris (pH 6.9)	250.0μl
1.0M $MgSO_4$	50.0μl
0.1M Dithiothreitol	5.0μl
0.1M dATP	3.0μl
0.1M dGTP	3.0μl
0.1M dTTP	3.0μl
Sterile water	186.0μl

STRIP A (1L)

20x SSC	10.0ml
10% SDS	10.0ml